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BIOLOGICAL BULLETIN

VITALITY OF THE GAMETES OF *CUMINGIA TELLINOIDES.*

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From the Marine Biological Laboratory, Woods Hole, Mass.

Cumingia tellinoides is a small lamellibranch mollusk found abundantly in the Woods Hole region. Its breeding season extends over a period of three or three and a half months, beginning early in June. The production of eggs is continuous and spawning by each mature individual occurs two or three times during the course of the breeding season. Eggs may be had in great abundance during this time. One female may shed 300,000 eggs, although the average number extruded is estimated to be between 100,000 and 200,000. They are expelled into the sea water where they are fertilized.

The present paper is a report on experiments to determine the vitality of the gametes, and more especially the longevity of the unfertilized eggs. It may be assumed that when eggs are released from the ovary, they are normal. From the time of liberation, deterioration begins and if not fertilized they ultimately go to pieces by fragmenting in a characteristic manner. The extensive work of Goldforb indicates that some deterioration takes place in sea urchin eggs while stored in the ducts of the gonad before extrusion. This may also be true of *Cumingia*, since two clear cases of physiologically poor lots of eggs have been found among hundreds of normal ones. Deterioration o both gametes of *Cumingia* and of *Arbacia* is rapid after extrusion into sea water.

VITALITY OF THE EGGS.

The eggs used in these experiments were obtained by isolating sexually mature individuals in small stender dishes filled with sea

water into which the eggs were shed. The eggs, so collected in one dish, may therefore be conveniently referred to in the description of the experiments as a "batch" or a "lot" of eggs. The reference in all cases is to the eggs of a single female.

During the summer of 1926 many batches of eggs were kept without fertilization to test their longevity. During the progress of the experiments a few eggs were taken from the dishes from hour to hour and fertilized as a test of their physiological condition and of the rate of deterioration as indicated by decreasing percentage of fertilization. The experiments reported here deal principally with the longevity of the unfertilized eggs as indicated by the time of disintegration.

Approximately two per cent. of the eggs of most females are defective at spawning and fragment within four or five hours. The other ninety-eight per cent. remain intact and capable of development for longer periods. The poorest lot of eggs when tested at a temperature of 20° to 22° centigrade went to pieces completely in six hours, and it was not uncommon for 98 per cent of the eggs of some females to fragment in from eight to nine hours. The great majority of the lots of eggs fragmented in from nine to fifteen hours, the average being between ten and twelve hours. When tested at 18.5° to 20° C., on the other hand, the most vigorous batches of eggs, remained intact from fifteen to twenty hours, that is, the eggs retained their normal appearance for that length of time. It was shown that a majority of the eggs either refuse to develop or develop abnormally, when fertilized shortly before fragmentation. However, a few normal embryos come from the oldest eggs, even when the percentage of fertilization is greatly reduced. When a lot of eggs is fertilized after fifty per cent. of them have fragmented, it not infrequently happens that most of those which remain intact fertilize and cleave. However, most of them die in cleavage or gastrula stages and few develop into normal embryos. The oldest lots of eggs to develop normally were ten and twelve hours old and rarely fifteen hours old. These all showed 95 to 98 per cent. fertilization and normal development.

The extreme limits of longevity of the unfertilized eggs are therefore found to be six to twenty-six hours, and the limits of

fertilization and normal development in the best lots of eggs are ten to fifteen hours. These figures are for temperatures normally experienced by the eggs of the species, or 18.5° to 22° C. They therefore represent the normal longevity of the eggs.

The statement by Morgan that the eggs of *Cumingia* will not stand rough handling is misleading. They are injured by centrifuging but there is abundant evidence that they develop normally under ordinary laboratory manipulation.

Tables I., II. and III. deal with various phases of the longevity and vitality of the eggs and include representative experimental data.

Individual Variation.

After any particular lot of eggs begins to fragment, three or four hours elapse before all or 98 per cent. have fragmented. The variation in longevity within a single group is, therefore, approximately four hours. For example, if 10 per cent. of the eggs have fragmented at twelve hours, the average expectancy of fragmentation for the remainder would be 30 per cent. in thirteen hours, 50 per cent. in fourteen, 70 per cent. in fifteen, and 95 to 98 per cent. in sixteen hours. Two per cent. may remain intact indefinitely. The rate of fragmentation is shown in Table I., as is also an actual comparison of the longevity of five lots of eggs from five females studied under identical conditions. Tables II. and III. show the same thing in slightly different form.

When fresh eggs are fertilized by fresh sperm the percentage of cleavage is usually between 97 and 100 per cent. However, a few lots of eggs gave from 89 to 95 per cent. cleavage. One lot of physiologically poor eggs was observed which when fertilized with physiologically good sperm gave only 20 to 30 per cent. cleavage. This sperm when used to fertilize other lots of eggs gave from 97 to 100 per cent. cleavage showing that failure to cleave on the part of these eggs was not due to defective sperm. This lot of physiologically poor eggs was slimy from the first and showed a tendency to cling to the containing dish. This together with one other lot of defective eggs may be regarded as confirmation of Goldforb's contention that eggs at the time of spawning may show all the symptoms characteristic of aging

TABLE I.

VITALITY OF *Cumingia* EGGS.

Record of five lots of eggs spawned by five females on August 16 and kept unfertilized at 20° C. The vertical columns, when read from top to bottom, give the result of eleven examinations that were made with the microscope at approximately hourly intervals in order to ascertain the time that elapsed before fragmentation began in each lot of eggs as well as the rate of fragmentation. The percentages refer to the number of eggs that remain normal in appearance. From three to five hundred eggs were counted in each examination.

	♀ No. 1.	♀ No. 2.	♀ No. 3.	♀ No. 4.	♀ No. 5.
After 5½ hrs.	100% normal	97% normal	100% normal	100% normal	100% normal
After 6½ hrs.	70% normal	100% normal	100% normal	100% normal	100% normal
After 8 hrs.	20% normal	5% normal	100% normal	100% normal	100% normal
After 9 hrs.	0% normal	0% normal	100% normal	100% normal	98% normal
After 10 hrs.			100% normal	100% normal	98% normal
After 11½ hrs.			100% normal	98% normal	90% normal
After 12 hrs.			100% normal	98% normal	80% normal
After 13 hrs.			98% normal	95% normal	50% normal
After 14 hrs.			95% normal	85% normal	30% normal
After 14½ hrs.			90% normal	70% normal	2% normal
After 15 hrs.			80% normal	50% normal	0% normal

Comment.—Eggs from lots 3, 4 and 5 were inseminated with freshly shed sperm after 11½ hours. The percentage fertilization which resulted was estimated to be 90 to 95 per cent. and abundant normal larvæ developed. Many eggs are apparently normal for 12 hours after extrusion. Eggs from lot 3 were fertilized 13 hours after extrusion which gave 75 per cent. cleavage and normal development. It appears that there is great variation in the vitality of the eggs of different females. These five lots spawned by five different females during the same hour were treated alike. One fragmented in seven hours, one in eight hours, one in fourteen hours, one in sixteen hours and it may be predicted in the light of former experience that the other would have fragmented completely in seventeen or eighteen hours if the experiment had been continued. No. 1 began to fragment in six hours. This gives the range of variation in longevity among the eggs from the five females as six to eighteen hours and normal fertilization for twelve hours in the best of the five. These five lots of eggs are substantially typical and may be considered as representative, although some are considerably longer lived than those used in this experiment. At least twenty such experiments were carried out during the season. The extreme limits of longevity were found to be from five to twenty-six hours. Only one or two per cent. of the best lots of eggs remained intact after the expiration of that time. It is interesting to compare this with the egg of *Arbacia* which, according to Goldforb and others, has a longevity of 40 to 48 hours.

TABLE II.

NORMAL VARIATION IN PERCENTAGE CLEAVAGE IN THE EGGS OF *Cumingia tellinoides*.
Temperature 22° C.

August 4. Freshly spawned eggs from ten females were fertilized by the sperm of one male to show the initial variation in the percentage of cleavage of eggs of this species. This table also shows the effect of aging. It is interesting to note that after 90 to 95 per cent. of the eggs have fragmented those remaining intact may fertilize and cleave. (Compare Tables II. and III.) All eggs normal and fragmented, are included in the calculation of the percentages.

	After 2 Hrs.	After 8 Hrs.	After 11 Hrs.	After 14 Hrs.	After 16 Hrs.
Female No. 1.....	98% cleavage	98% cleavage	93% cleavage	10% cleavage	0% cleavage
Female No. 2.....	98% cleavage	98% cleavage	40% cleavage	0% cleavage	0% cleavage
Female No. 3.....	92% cleavage	10% cleavage	0% cleavage	0% cleavage	0% cleavage
Female No. 4.....	97% cleavage	70% cleavage	71% cleavage	50% cleavage	2% cleavage
Female No. 5.....	98% cleavage	7% cleavage	0% cleavage	0% cleavage	0% cleavage
Female No. 6.....	96% cleavage	97% cleavage	92% cleavage	60% cleavage	20% cleavage
Female No. 7.....	99% cleavage	98% cleavage	96% cleavage	2% cleavage	0% cleavage
Female No. 8.....	95% cleavage	95% cleavage	95% cleavage	40% cleavage	10% cleavage
Female No. 9.....	88 to 90% cleavage	30% cleavage	20% cleavage	1% cleavage	0% cleavage
Female No. 10.....	30% cleavage	0% cleavage	0% cleavage	0% cleavage	0% cleavage

eggs. The first vertical column of Table II. shows the usual range of variation in freshly spawned eggs in respect to percentage fertilization and cleavage.

TABLE III.

LONGEVITY OF THE EGGS OF *Cumingia tellinoides*, TEMPERATURE 22° C.

The eggs of the ten females compared in Table II. in respect to percentage fertilization are here compared as to longevity and time of fragmentation or disintegration. It will be noted that the time of reduction in cleavage is almost synchronous with the time of fragmentation. This relationship does not always hold because in numerous experiments the percentage of cleavage fell off before fragmentation. In other words, if the reduction in cleavage due to aging and the rate of fragmentation were plotted as curves they would not as a rule be superimposed or parallel.

	Percentage of Fragmentation with Aging.			
	After 7 Hrs.	After 11 Hrs.	After 15 Hrs.	After 16 Hrs.
Female No. 1.....	0%	0%	50%	100%
Female No. 2.....	0%	45%	100%	100%
Female No. 3.....	75%	100%	100%	100%
Female No. 4.....	0%	2%	72%	90%
Female No. 5.....	73%	100%	100%	100%
Female No. 6.....	0%	0%	40%	50%
Female No. 7.....	0%	0%	99%	100%
Female No. 8.....	0%	0%	12%	60%
Female No. 9.....	0%	7%	100%	100%
Female No. 10.....	100%	100%	100%	100%

Note.—Many of the eggs of lots 4, 6 and 8 lived beyond sixteen hours. The other lots of eggs had fragmented completely after sixteen hours.

VITALITY OF THE SPERMATOZOA.

The work of Gemmill ('00) and F. R. Lillie ('15) has shown that variations in the concentration of sperm suspensions of *Arbacia* make a great difference in the duration of their fertilizing ability. The writer accordingly used several sperm dilutions in order to learn whether the same phenomena are exhibited by the sperm of *Cumingia*.

Spermatozoa shed by a mature male *Cumingia* of average size in 30 cc. of sea water was estimated by actual measurement to be a 2 to 3 per cent. suspension.[1]

[1] The method of measuring the percentage was to kill the spermatozoa by the addition of formalin and after settling they were measured en masse. From this the calculation of the concentration of the original suspension was a simple matter.

This concentrated suspension, though at best somewhat variable, was used as a standard suspension from which various dilutions were made. It was found possible to select suspensions of approximately the same strength and this is a matter of more importance than that the exact percentage be known.

Two drops[2] of standard sperm suspension when added to 25 cc. of sea water was estimated to be a 1/500 to 1/750 per cent. suspension; two drops in 50 cc. of sea water was considered to be a 1/1000 to 1/1500 per cent. suspension; one drop in 50 cc. of sea water makes a 1/2000 to 1/3000 per cent. suspension, etc. The last named suspension when fresh is adequate to fertilize one hundred per cent. of the eggs whereas greater dilutions sometimes gave only partial fertilization. What was estimated to be a 1/4000 to 1/6000 per cent. suspension gave from eighty to one hundred per cent. fertilization and usually one hundred per cent. All of these suspensions were used and also the same percentage suspensions in larger quantities of sea water.

The method of studying the relative longevity of these various dilute sperm suspensions was to make up, by the proper dilutions, several dishes of each from freshly shed sperm. To these fresh eggs were added in turn at hourly intervals until the suspensions no longer gave fertilizations. It was shown in general that the weakest suspensions die first. Tables 4 and 5 show that the longevity of the spermatozoa depends largely upon the degree of concentration. Even a suspension of 1/500 per cent. shows some preserving effect in that spermatozoa live for a longer time in this concentration than in greater dilutions. There is little difference between a 1/2000 and a 1/6000 per cent. suspension, and these no doubt represent natural conditions so far as longevity is concerned.

In work reported at this time the writer had in mind to study the longevity of gametes under natural conditions. In general the longevity of the sperm in the most dilute suspensions is somewhat less than that of the eggs. In a few cases using suspensions of 1/500 per cent. approximately ninety per cent. normal embryos developed from sperm and eggs that were

[2] The same pipette was used in making all dilutions of an experiment and one giving approximately one cc. per 20 drops.

twelve hours old and in numerous other cases at nine and ten hours. As a rule when sperm suspensions of 1/2000 to 1/3000 per cent. are used very few fertilizations occur after nine or ten hours. Numerous experiments show that spermatozoa begin to die after three and one half or four hours. The indication therefore is that a majority of the spermatozoa under natural conditions live from four to nine hours.

TABLE IV.

LONGEVITY OF THE SPERM OF *Cumingia* IN VARIOUS DILUTIONS AS SHOWN BY THE PERCENTAGE OF FERTILIZATIONS THAT THEY GIVE WITH FRESH EGGS (JULY 25).

Age of the Suspension Tested.	1 Drop in 100 Cc. Sea Water 1/4000 to 1/6000%.	1 Drop in 50 Cc. Sea Water 1/2000 to 1/3000%.	4 Drops in 50 Cc. Sea Water 1/500 to 1/750%.	8 Drops in 50 Cc. Sea Water 1/250 to 1/500%.
2 hours	99%+	99%+	100%	100%
3½ hours	96%	95%	97%	96%
7 hours	17%	24%	99%	97%
10 hours	2%	8%	63%	72%

For this experiment the spermatozoa used were all from the same male. The eggs were from three females and were not over three hours old when used. Controls showed them to be practically 100 per cent. normal eggs.

TABLE V.

LONGEVITY OF THE SPERM OF *Cumingia* IN VARIOUS DILUTIONS TESTED ON JULY 23.

Sperm all from one male.

Age of Sperm Suspension Used.	1 Drop in 50 Cc. Sea Water 1/2000 to 1/3000%.	2 Drops in 50 Cc. Sea Water 1/1000 to 1/1500%.	4 Drops in 50 Cc. Sea Water 1/500 to 1/750%.	6 Drops in 50 Cc. Sea Water 1/300 to 1/500%.
2½ hours	84% cleaved	100% cleaved	100% cleaved	99%+ cleaved
4½ hours	35% cleaved	65% cleaved	75% cleaved	80% cleaved
7½ hours	15–20% cleaved	30% cleaved	50–60% cleaved	85–87% cleaved
9 hours	0% cleaved	0% cleaved	2% cleaved	35–40% cleaved

This table shows that sperm in 1/2000 to 1/3000 per cent. suspension died in nine hours. Other experiments have shown that from ten to forty per cent. of the spermatozoa in these most dilute suspensions often survive for 10 to 12 hours. Table IV. is more typical in this respect, but even in this case the percentage of fertilization is below the average. The point that is shown clearly is that spermatozoa die first in the most dilute suspensions and that a suspension of 1/500 to 1/750 per cent. suspension preserves the life of the sperm beyond their normal life in the open sea.

When the work of Gemmill and Lillie is taken into consideration it is apparent that Goldforb's experiments on the aging of spermatozoa do not represent normal conditions. He apparently relied upon concentrated dry sperm which are known to age much more slowly than sperm kept under natural conditions. The aging of sperm in concentrated suspensions has no particular significance. His work on the eggs is certainly not open to the same criticism but I should question his interpretation and results on the aging of spermatozoa. The work of the other two authors and this present work show that the life of spermatozoa in dilute suspensions is brief.

The statement by Lillie that the spermatozoa of *Arbacia* lose ability to fertilize eggs in a few minutes in dilute suspensions is not verified for *Cumingia* sperm, although the theory that they gradually lose something to the water which is essential to fertilization and which ultimately renders them incapable of bringing about the fertilization reaction may very well be true.

It has been my observation that spermatozoa that are able to swim actively are capable of fertilizing eggs; and that they do not lose the ability to initiate the fertilization reaction after a few minutes as reported by Lillie for *Arbacia*. Up to the present time no attempt has been made to learn whether or not the spermatozoa of *Cumingia* contain an activating substance, essential to fertilization. In many ways the spermatozoa of *Cumingia* behave as those of *Nereis* and *Arbacia* do, but if they contain a sperm receptor (Lillie, 15) preliminary experiments indicate that it is dissipated in the sea water more slowly than in the cases investigated by Lillie. Experiments on this question are in progress, as well as investigations of the cause of excessive polyspermy. These experiments are designed for comparison of the gametes of *Cumingia*, *Chætopleura* and *Hydroides* with the work of Goldforb and Gemmill on sea urchin eggs and with Lillie's work on the spermatozoa of *Nereis* and *Arbacia*.

Activation of Spermatozoa.

Spermatozoa, after having apparently lost their vitality, may be revived. When placed in a dish with eggs they become activated and swim vigorously. The activation of sperm in the

presence of eggs was observed repeatedly. The stimulus from the eggs or egg water is evident almost instantly, but not all of the spermatozoa are so activated. I interpret this to mean that most of the quiescent spermatozoa are already dead or weakened beyond the possibility of functioning.

The phenomenon of activation of apparently spent sperm by the exudations from eggs is interesting and not uncommon. The physiological value of this activation is evident although the real cause is obscure. Lillie (13) shows that the spermatozoa of *Nereis* and *Arbacia* are positively chemotropic to weak acids and to egg secretions and are apparently stimulated by them.

Concentrated Sperm Suspensions.

It was learned that spermatozoa in concentrated suspensions retain their vitality for very long periods. Under such conditions they may swim from twenty-four to thirty-six hours and give 90 to 100 per cent. fertilization and normal development. They retain sufficient life for four days to show occasional contractions visible under a compound microscope. It is evident therefore that there is some protective element in this unnatural concentration. Cohn claims that it is carbon dioxide or hydrogen ion concentration. Dry sperm of fishes has the same extended longevity although possibly from different causes, not having had the initial stimulus to swim and use up its limited store of energy.

Discussion.

Goldforb expresses the belief that deterioration of eggs begins at the time of maturation while they are still stored in the gonads. He attributes the great variability of sea urchin eggs to the differences in time that they remain in storage before spawning takes place. I find a similar variation in the vitality of eggs of *Cumingia*, but it is noteworthy that maturation does not take place in the eggs of this species until after the entrance of the spermatozoön which occurs after extrusion into the sea water. Deterioration in the gonad in this case, therefore, could not be due to maturation. The fact that eggs vary so much in their longevity leads to the belief that some deterioration takes place before extrusion and before maturation. It is hardly likely that

the great difference in vitality that experiment reveals is due entirely to natural variability. The eggs are stored in the gonads and their ducts for some time before extrusion and those that are stored longest may very well show physiological deterioration. Two cases of eggs which showed all the symptoms of aging at spawning verify Goldforb's contention on this point. At the present time I am unable to state whether the considerable variability in the longevity of *Cumingia* eggs is due principally to normal variability or is in part due to physiological deterioration while in storage. Cases of low percentage fertilization in the eggs of *Cumingia* are rare. As a rule they give 97 to 100 per cent. cleavage, although the longevity may be more variable.

Summary.

I. The average longevity of *Cumingia* eggs when kept at a temperature of 20° to 22° C. is 10 to 12 hours as judged by time of fragmentation and ability to give a high percentage of normal embryos. The average longevity at 18.5° to 20° C. is 12 to 15 hours.

II. Approximately two per cent. of any lot of eggs may be defective as shown by their early fragmentation which occurs long before the rest.

III. The outstanding fact is the wide range of variation in the vitality and longevity of *Cumingia* eggs. The eggs of a single individual vary by four hours or approximately 25 per cent., while the eggs of different females vary in their longevity from six to twenty-six hours or over 400 per cent.

IV. Eggs in rare instances show deterioration at spawning, apparently due to long-time storage in the ducts of the gonads. Most lots of eggs when freshly spawned give from 97 to 100 per cent. cleavage and normal development.

V. The longevity of the best sperm in suspensions of 1/400 to 1/500 per cent. is 10 to 12 hours as judged by functional activity and ability to give 90 to 100 per cent. fertilization and normal development. In sperm suspensions of 1/2000 to 1/3000 per cent. it is 4 to 7 hours, but from 30 to 50 per cent. fertilization may frequently be expected from suspensions 7 to 12 hours old and 1 to 5 per cent. fertilization from suspensions 12 to 20 hours old.

VI. Spermatozoa in concentrated suspensions of 1 to 3 per cent. retain their vigor for many hours. They frequently give 90 to 100 per cent. fertilization after twenty-four to thirty-six hours, and some individual spermatozoa live for four days, still showing faint contractions of the tail at intervals. This preservation of the life of the spermatozoa is attributed to the presence in the water of CO_2 produced by the activity of the spermatozoa, or to hydrogen ion concentration.

VII. Spermatozoa after becoming quiescent show activation in the presence of eggs or egg water.

This paper was read by title before the American Society of Zoölogists at the meeting in Philadelphia, December, 1926. An abstract was printed in the *Anatomical Record.*

BIBLIOGRAPHY.

Cohn, E. J.
'16 The Relation between the Hydrogen Ion Concentration of Sperm Suspensions and their Fertilizing Power. Anat. Rec., Vol. II, p. 530.

Goldforb, A. J.
'17 Variability of Eggs and Sperm of Sea-Urchins. Publication No. 251 of the Carnegie Inst. Washington, D. C., p. 71.

Goldforb, A. J.
'18 Effects of Aging upon Germ Cells and upon Early Development. BIOL. BULL., Vol. 34, p. 372.

Gemmill, J. F.
'00 On the Vitality of the Ova and Spermatozoa of Certain Animals. Jour. of Anat. and Physiol., Vol. 34 (N.S., Vol. 14), p. 163.

Lillie, F. R.
'13 The Behavior of the Spermatozoa of *Nereis* and *Arbacia* with Special Reference to Egg-extractives. Jour. Exp. Zoöl., Vol. 14, p. 515.

Lillie, F. R.
'15 Analysis of Variations in the Fertilizing Power of Sperm Suspensions of *Arbacia*. BIOL. BULL., Vol. 28, p. 228.

Lillie, F. R.
'15 Sperm Agglutination and Fertilization. BIOL. BULL., Vol. 28, p. 18.

Nelson, T.
'21 Report of The Biologist, N. J. Agr. Exp. Sta., year ending June 30, 1921, p. 297.

CONDITIONS DETERMINING THE ORIGIN AND BEHAVIOR OF CENTRAL BODIES IN CYTASTERS OF *ECHINARACHNIUS* EGGS.[1]

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INTRODUCTION.

Statement of the Problem Concerning the Origin of Central Bodies.

In every cell generation there are differentiated the various cell components such as chromosomes, plastids, Golgi bodies, chondriosomes, central bodies, secretory droplets and similar elements. In the case of certain ones, such as chromosomes, the synthetic processes producing new units occur only by the growth and division of preëxisting bodies of the same kind. Such a component has genetic continuity from cell to cell as an individualized body, appearing to play some rôle in its own formation. Its origin may therefore be termed autogenic or self-perpetuating. In contrast to this, the synthetic processes forming other components, such as secretory droplets, are not localized about preformed structures of the same kind. Such a component has no

[1] The experiments were performed at the Mt. Desert Island Biological Station, Maine, 1923, and at the Marine Biological Laboratory, Woods Hole, Massachusetts, 1926.

genetic continuity as an individualized body and its origin is commonly described as occurring de novo.

Central bodies[2] have special interest with reference to this problem of protoplasmic differentiation, since in some cases they arise by the growth and division of preëxistent central bodies, while in other instances they apparently are formed de novo. In some cells they persist from one cell division to another, maintaining their identity as individualized structures during the vegetative period when the mitotic mechanism, of which they appear to be the formative foci, has disappeared. At a certain time in the cell cycle they divide and the daughter cells receive central bodies that have genetic continuity with that of the mother cell. For example, in spermatogenesis, the central body of the sperm can frequently be traced as a self-perpetuating individualized structure to the central body of the primary spermatocyte.

On the other hand there is also much evidence for a de novo origin of central bodies. In higher plants where centers are absent, blepharoplasts which have the characteristics of central bodies appear de novo in the final divisions of the germ cells. In animal fertilization it has yet to be proved that the central body of the sperm aster has genetic continuity as an individualized structure with the central body of the sperm, except in a few forms such as *Cerebratulus* (Yatsu, '09), and in most cases its formation may be de novo. When eggs are artificially activated in such a way that cytasters are formed, a study of sectioned material shows that they have central bodies (Morgan, '96, '99, '00; Wilson, '01; Hindle, '10; Herlant, '19; Fry, '25*b*; Tharaldsen, '26; etc.). Since cytasters appear simultaneously in large numbers and not by division the central bodies are apparently formed de novo.[3]

[2] The term central body is used throughout this paper in its broad non-committal sense, denoting any differentiated structure present at the central region of an aster, exclusive of the inner ends of the rays (and the possible presence of chromatin). The term involves no implications as to whether or not the central body is composed of a granule, the centriole, and a surrounding zone, the centrosome, either of which may be present or absent in various organisms, and both of which may show many modifications (Wilson, '25, pp. 30 and 673).

[3] For a detailed discussion of the literature on this subject see Wilson "The Cell in Development and Inheritance," 1925, especially Chapter IX.

The idea that all central bodies are self-perpetuating structures received an impetus due to the early theory of fertilization developed by Boveri and Van Beneden. They assumed that the chief event in fertilization is the introduction by the sperm into the egg of a preformed division center and hence that the central body of the sperm aster has genetic continuity as an individualized structure with that of the sperm (Boveri, '01). So generally accepted was this assumption that when artificial parthenogenesis was discovered, where the aster appears about the egg's nucleus, it was taken for granted that the egg nucleus must therefore have such a preformed central body that is ordinarily quiescent under the normal conditions of fertilization. To explain cytasters according to this theory it was necessary either to regard them as "pseudoasters," assuming that if they do not arise about preformed centers they are not "true asters," or to assume that central bodies lie scattered through the cytoplasm as a result of hypothetical rapid divisions of the egg's hypothetical division center (Meves, '02; Wassilieff, '02; Petrunkewitsch, 04). These ideas which are without proof are only of interest to illustrate the strength of the underlying assumptions which are still generally accepted, *i.e.*, that central bodies have genetic continuity as individualized structures, and that asters ordinarily arise about such units on the theory that the central body is the persistent element of the astral mechanism acting as the formative focus.

A possible explanation of the seemingly conflicting evidence that in some cases central bodies have an autogenic origin and in others a de novo one has been proposed by Wilson. He suggests that central bodies which seem to arise de novo, may actually be self-perpetuating structures that are submicroscopic during most of their history; they grow to a visible size only at certain times when they give the impression of having a de novo origin. This suggestion is in harmony with the general assumption that central bodies are individualized structures maintaining genetic continuity and giving rise to asters. He states: ". . . the cytologist is led on to the conclusion that the ultramicroscopical dispersed particles of the hyaloplasm may be as highly diversified chemically as are the visible formed bodies;

that they may be of all orders of magnitude; and that it is they which constitute the sources, or at least the formative foci, of those larger formed bodies that we have so often, but erroneously, assumed to arise de novo. For my part, I am disposed to take a final step by accepting the probability that many of these particles (I do not say all) as if they were ultra-microscopical plastids, may have a persistent identity, perpetuating themselves by growth and multiplication without loss of their specific individual type" ('23, p. 28). "Could we accept such a view we could more readily meet some puzzling difficulties such, for example, as the apparent contradiction between the origin of a centriole de novo, and its origin by division of a preëxisting body of the same kind" ('25, p. 721).

Morgan ('96, '99, '00) has made the most careful study of the relationship between central bodies and the structure of the surrounding cytasters. As a result of his observations he raised certain questions, in sharp contrast to the generally accepted ideas, that have since been largely ignored: "Is this central deeply staining mass a distinct body, or only the innermost fused ends of the rays; or is it only a product of the reagent?" ('99, p. 477). The present study is an attempt to resolve these conflicting hypotheses concerning the origin and behavior of central bodies in cytasters.

Types of Evidence Valid for Proving a Self-perpetuating or a de novo Origin of a Cell Component.

The behavior of a cell component in hybrids yields the only conclusive evidence concerning the nature of its origin. For example, in hybrids the paternal chromosomes and their constituent genes continue to perpetuate themselves and produce their effects in the protoplasm of another species. This conclusively proves that they are autogenic, playing an important rôle in their own synthesis. It demonstrates that under normal conditions, within the protoplasm of their own species, they are not passively produced by more active elements, otherwise they would not continue to grow and divide in an alien protoplasm. There is similar evidence for the autogenic origin of certain plastids. When species with different plastids are crossed, the

paternal type continues to perpetuate itself in a cytoplasm that normally contains a different kind of plastid. The autogenic origin of other cell components has not been subjected to this conclusive proof of their capacity to perpetuate themselves by growth and division in the protoplasm of another species. Such a study of central bodies, in reciprocal crosses between *Arbacia* and *Echinarachnius*, is now in progress, a report of which will be presented in a later paper.

After reviewing the facts concerning certain plastids, Morgan ('26) states: "It may not appear far fetched to assume that there may be other bodies in the cytoplasm that grow and divide and, by extension, it might not seem too extravagant to assume that protoplasm itself (except for its secretion products) consists of units that grow and divide and are inherited." He calls attention, however, to the fact, very significant in this connection, that when two species are crossed reciprocally they produce similar hybrids. If some of the cytoplasmic components autonomously perpetuate themselves, maintaining their characteristic form, then in reciprocal hybrids the adults should show cytoplasmic differences. In one cross the cytoplasm with any self-determining units is derived from one species, and in the opposite cross it comes from the other. Since the cytoplasm of the adults is similar in both, despite its differing sources in the two crosses, this shows that its components generally are not autogenic in such a manner as to maintain their identity. It indicates that the synthetic processes are under the control of the nucleus producing similar results in both cases. It is generally to be expected, therefore, with some exceptions such as certain plastids, that cytoplasmic components do not perpetuate themselves autogenically maintaining their characteristic identity.

A component's power of growth and division, therefore, within the protoplasm of another species is primary evidence for its autogenic origin; its power of growth and division in the protoplasm of its own species constitutes a second grade of evidence indicating such an origin. Were there no genetic evidence from hybrids as to the self-perpetuating nature of chromosomes and certain plastids and were their behavior studied only in the cells in which they normally occur, the fact that they produce

new units by the growth and division of similar preëxisting ones indicates an autogenic origin. When centrioles of secondary spermatocytes are produced by the growth and division of centrioles of primary spermatocytes, this is evidence that at least they localize the synthetic processes forming new centriole substance and therefore are autogenic to that extent. In so far as there is evidence for the formation of chondriosomes, Golgi bodies and vacuoles from similar preëxisting units, to that extent is there probability of their autogenic origin.[3]

Wilson ('01) describes the division of central bodies in dividing cytasters of artificially activated eggs of *Toxopneustes*, noting the significance of this with reference to the self-perpetuating nature of such central bodies. Fry ('25*b*) studied the behavior of cytasters in nucleated and enucleated *Echinarachnius* eggs in both living and fixed material, and found division only in those instances when they secondarily established connection with chromatin. Tharaldsen ('26) found the same situation in cytasters of *Asterias*. Although a cytaster may divide, this has no significance concerning the origin of its central body unless it is conclusively proved: (1) that the central body is an individualized structure independent of the rays, and (2) that its division initiates, and is not just a result of, the division of the surrounding cytaster. Since both of these points are unproved, and since the present study brings strong evidence against the former, the facts concerning the division of central bodies in dividing cytasters are in too uncertain a state to use them as evidence of an autogenic origin.

The foregoing types of evidence concerning a component's self-perpetuating nature, based upon its power of division, whether within the protoplasm of another species or within its own, assumes that it is microscopically visible throughout its history. In those components where new units do not visibly arise by division, but apparently are formed de novo, evidence concerning their origin becomes more difficult to secure. As previously noted, they actually may be self-perpetuating bodies that are submicroscopic during part of their history, becoming visible only at certain times. "Manifestly it is quite illogical to affirm an origin de novo of any formed body because it first becomes visi-

ble at a particular enlargement, even the greatest at our present command" (Wilson, '23, p. 24). Data can be obtained, however which may strongly support either an autogenic origin or a de novo one. If a component is obviously the product of another component; if its behavior is invariably correlated with certain changes in surrounding structures, then the presumption is very strong that it is produced by them and has a de novo origin. If, on the other hand, it is obviously not the product of surrounding structures but rather is their formative and controlling factor; if it passes through a certain definite cycle which is to some degree independent of modifications in surrounding structures; such facts, depending upon the details of the evidence, may indicate its self-perpetuating nature. Data of this type were secured in the present study.

Experiments.

This investigation deals with the origin and behavior of central bodies in cytasters of artificially activated eggs of *Echinarachnius parma*, to ascertain whether they are de novo structures produced by the cytasters, or whether they are individualized structures having an identity independent of the surrounding cytaster which forms about them, and have further characteristics indicating a self-perpetuating origin. The experiments are arranged as follows: (1) keeping the environmental factors of activation constant, to observe the various effects of different fixatives upon rays and central bodies; (2) keeping the fixative constant, to observe the various effects of modifications of environmental factors such as temperature and osmotic pressure upon rays and central bodies; (3) keeping both fixative and environmental factors constant, to observe the effects of various intervals during the astral history upon rays and central bodies.

The Effects of Various Fixatives upon Rays and Central Bodies of Cytasters (Chart I.).

The eggs for this group of experiments, as well as the others to be reported later, were obtained by removing the oral surface of the animals and securing eggs from those individuals where they appeared upon the exposed ovaries. The activation techni-

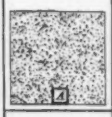
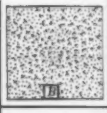
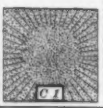
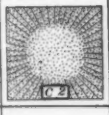
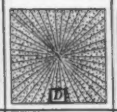
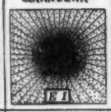
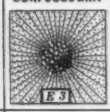
EXPERIMENT NUMBER	FIXATIVE		PERCENTAGES OF		
	NAME [THOSE CONTAINING ACETIC ACID ARE MARKED BY AN ASTERISK]	FORMULA	NO RAYS NO CENTRAL BODY  A	VAGUE RAYS NO CENTRAL BODY  B	DISTINCT RAYS NOT EXTENDING TO CENTER NO CENTRAL BODY [CENTRAL AREA STAINED SAME AS RAY AREA]  C
1	* FLEMMING - STRONG	1% Chromic acid 15 pts 2% Osmic acid 4 pts Acetic acid 1 pt			
2	FLEMMING - STRONG WITHOUT ACETIC	1% Chromic acid 15 pts 2% Osmic acid 4 pts		70	
3	* FLEMMING - WEAK	Strong Flemming 1 pt Water 3 pts		12	17
4	FLEMMING - WEAK WITHOUT ACETIC	Strong Flemming without Acetic acid 1 pt Water 3 pts		75	
5	OSMIC ACID	.025% Osmic acid		100	
6	CORROSIVE SUBLIMATE COLD - ALKALINE	Saturated solution - $HgCl_2$ - Cold - Made alkaline with NaOH		100	
7	CORROSIVE SUBLIMATE COLD - ACID	Saturated solution $HgCl_2$ - Cold - Has acid reaction	50	50	
8	CORROSIVE SUBLIMATE HOT - ACID	Saturated solution $HgCl_2$ Heated to 90°C. Has acid reaction	100		
9	* SUBLIMATE ACETIC 1%	Sat. solution $HgCl_2$ - 99 pts Acetic acid 1 pt.	15	25	2
10	* SUBLIMATE ACETIC 5%	Sat. solution $HgCl_2$ - 95 pts Acetic acid 5 pts	38	33	
11	* SUBLIMATE ACETIC 10%	Sat. solution $HgCl_2$ 90 pts Acetic acid 10 pts	15	50	2
12	* PICRO-ACETIC - STRONG	Sat. sol. Picric acid 1 pt Water 2 pts Acetic acid 5 %		30	25
13	* PICRO-ACETIC - WEAK	Picro - Acetic - Strong 1 pt Water 3 pts		20	15
14	* BOUIN	Sat. sol. Picric acid 75 pts Formol 25 pts Acetic acid 5 pts		10	10
15	FORMOL 5% ALKALINE	5% Formol made alkaline with $MgCO_3$		100	
16	FORMOL 5% ACID	5% Formol. Has acid reaction	14	86	
17	ALCOHOL 100%	100% Alcohol	25	75	
18	ALCOHOL 35%	35% Alcohol		85	15
19	* ZENKER	Potassium dichromate 2 gm Sodium sulphate 1 gm Water 95 cc Mercuric chloride 5 gm Acetic acid 5 cc		80	
20	ZENKER WITHOUT ACETIC	Same as above without Acetic acid		100	
21	* CARNOY and LEBRUN	100% Alcohol 3 pts Acetic acid 1 pt Chloroform 1 pt Mercuric Chloride to saturation		5	20
22	CARNOY and LEBRUN WITHOUT ACETIC	Same as above without Acetic acid.	2	45	15
23	* PETRUNKEWITSCH	Water 500 pts 100% Alcohol 200 pts Acetic acid 90 pts Nitric acid 10 pts Mercuric Chloride to saturation		22	
24	PETRUNKEWITSCH WITHOUT ACETIC AND NITRIC	Same as above without Acetic and Nitric acids.		94	2

CHART I. THE EFFECTS OF VARIOUS FIXATIVES

Activation: same in all experiments; a slight over exposure of the optimum butyric-hypertonic technique, cf. footnote 4, p. 372. *Fixatives:* as listed. *Times of fixation:* at frequent intervals after activation, cf. footnote 5, p. 372. *Percentages of types of cytasters:* averages of detailed counts made at the intervals at which eggs were fixed, from twenty-five minutes until one hour after activation; each count based on about 100 cytasters chosen at random from a number of eggs; examples of such data for Fixatives 3, 10 and 13, shown in Chart III, p. 380.

TYPES OF CYTASTERS AND CENTRAL BODIES

	DISTINCT RAYS NOT EXTENDING TO CENTER NO CENTRAL BODY (CENTRAL AREA STAINED LIGHTER THAN RAY AREA)  C 2	DISTINCT RAYS EXTENDING TO CENTER NO CENTRAL BODY  D	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY VAGUELY DEMARKED GRANULAR  E 1	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED GRANULAR E 2	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED CORPUSCULAR  E 3	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED RETICULAR E 4
*1		54		46		
2		30				
*3	12	41	11	2	3	2
4		25				
5						
6						
7						
8						
*9		30	27	1		
*10		17	12			
*11		31	1	1		
*12	20	23	2			
*13	9	20	6	29		1
*14	15	56	1	6	1	1
15						
16						
17						
18						
*19		20				
20						
*21	75					
22	2	36				
*23	55	23				
24	4					

UPON RAYS AND CENTRAL BODIES OF CYTASTERS.

Illustrations: show only the central regions, cf. Fig. 2, p. 390. *Result:* when eggs containing similar cytasters produced by similar activation are fixed by various fixatives, central bodies are present only when the fixative coagulates the cytaster in such a manner that rays are distinct and reach the center. For further details cf. p. 369. *Secondary result:* with few exceptions, only fixatives containing glacial acetic acid (indicated by an asterisk) clearly fix rays. For further details cf. p. 375.

que used was that developed by Just ('19). An egg sample of each individual was given a preliminary test of their capacity to form clear membranes by treating them with butyric acid solution (2 cc. 1/10 *N* butyric acid plus 50 cc. sea water). Only those eggs were used that gave over ninety per cent. membranes.

An artificial activation for *Echinarachnius* that is optimum for producing the maximum number of normal embryos yields only about ten per cent. eggs with cytasters (Fry, '25*a*). The activation used in the present experiments, therefore, was a slight over exposure so as to produce about twenty-five per cent. eggs with cytasters to facilitate their study.[4] The eggs show an average of seven per cent. cytolysis, although there is considerable variation as to this in the different fixatives.

Cytasters of *Echinarachnius* arise about fifteen minutes after removal from the activating solutions. None divide except occasional ones that secondarily establish contact with chromatin, and if they are numerous, cleavage does not usually occur (Fry, '25*b*). Thus the majority of cytasters formed soon after activation remain unchanged for hours except as to modifications of their detailed structure which are studied by fixing samples of eggs at various intervals.

Eggs were fixed in twenty-four fixatives being placed in each at frequent intervals after activation.[5] They were sectioned 5 μ thick and stained in Heidenhain's hæmotoxylin. Various degrees of destaining, carefully studied with water immersion

[4] Experiments 1 to 4 inc. and 7 to 14 inc., Chart I, were activated with a 35 second treatment with butyric acid solution (2 cc. 1/10 *N* butyric acid plus 50 cc. sea water), followed by a 20 minute interval in sea water, followed by an 18 minute treatment with hypertonic sea water (5 cc. 2.5 *N* sodium chloride solution plus 50 cc. sea water) at 24° C. Experiments 5 to 6 inc. and 15 to 24 inc. were similarly activated with butyric acid solution, followed by a similar interval in sea water. In treating them with hypertonic sea water (same concentration), however, the eggs were divided into four lots and exposed 15, 20, 25 and 30 minutes respectively. The experiment was so arranged that all lots came out of the hypertonic solution at the same time, and then they were mixed. The temperature was 22° C. That the two groups received an essentially similar activation is shown by the fact that samples of both contained about the same percentages of various types of development at twelve hours after activation.

[5] Experiments 1 to 4 inc. and 7 to 14 inc., Chart I., were fixed at the following intervals after activation: 15 min., 17.5, 20, 22.5, 25, 27.5, 30, 35, 40, 45, 50, 55, 1 hr., 1 hr.-15 min., 1–30, 1–45, 2 hrs., 3, 4, 5. Experiments 5 to 6 inc. and 15 to 24 inc. were fixed at the following intervals: 10 min., 15, 20, 30, 45, 1 hr., 2, 3.

lenses, show no significant differences in results. Stains other than hæmotoxylin were not used as it has been generally found that they yield similar results, and hæmotoxylin is the accepted standard.

The percentages of the various types of cytasters and their central bodies shown for each fixative, Chart I., are averages of a number of detailed counts, one made for each interval when eggs were fixed, from twenty-five minutes to one hour after activation (cf. footnote 5, p. 372). This is the period during which cytasters are at their maximum development. Each count for each interval is based upon the study (at 750 × magnification) of about 100 cytasters and their central bodies selected at random from a number of eggs. Such detailed counts for Experiments 3, 10 and 13, Chart I., are shown in Chart III., p. 380.

Since sections were cut at 5 μ, and since the majority of cytasters are 10 to 15 μ in diameter, most of them are found in two or more serial sections. In the case of each cytaster only that section was studied that includes its mid-region (Fig. 1, *b*, p. 384), ignoring the others (Fig. 1, *a* and *c*). The drawings show only the central portion of such mid-sections. The peripheral area is omitted to save space on the charts since it yields no significant information concerning rays and central bodies not shown by the central area. An entire mid-section of a cytaster is illustrated, Fig. 2, p. 390; the dotted line makes clear how small a portion of the central region is shown in the chart illustrations. Although the drawings are necessarily diagrammatic they are an accurate representation of the various classes of cytasters and their central bodies. They have the following classification (cf. Chart I.):

Rays.	*Central Body.*	*Chart Symbol.*
None	None	A
Vague	None	B
Distinct, not extending to center:		
Central area stained same as ray area	None	C1
Central area stained lighter than ray area	None	C2
Distinct, extending to center	None	D
Distinct, extending to center	Vaguely demarked—granular	E1
Distinct, extending to center	Clearly demarked—granular	E2
Distinct, extending to center	Clearly demarked—corpuscular	E3
Distinct, extending to center	Clearly demarked—reticular	E4

When cytasters that are in a similar condition due to similar activation, are fixed with a variety of fixatives, central bodies are present only when the fixative coagulates the cytaster in such a manner that the rays are distinct and extend to the center. The fact that rays are distinct and reach the center does not guarantee the presence of central bodies, since many cytasters with such rays are without them (Type D). In the majority of such cases the rays are more delicate than in those cytasters having central bodies, where are usually found rays of maximum coarseness and clarity (Types E1, E2, E3 and E4). Central bodies are never present when rays are absent (Type A), or vague (Type B), or clear without reaching the center (Types C1 and C2). Differences in the size of cytasters, which may vary from 5 to 25 μ, do not affect this relationship between the presence of central bodies and distinct central rays, nor does the location in the egg which may be central or peripheral.

Within any one egg there are found only related types such as, A and B, or B and D, etc. Unrelated types do not occur together. There is a similar occurrence of related types among the different eggs of the same slide, fixed at a given interval after activation. Since the percentages of the different types shown for each fixative of Chart I. are averages of a number of detailed counts made at various intervals after activation, the variety of types found in the general average is usually greater than those found at any one interval.

Type A, without any rays, is easily identified as a cytaster since it is a granular area that stains darker than the surrounding cytoplasm, of the same size as a typical cytaster, and is frequently associated with those having vague rays. This rayless type is often found during the late history of asters when the rays frequently fade out completely.

In this series of experiments, concerning the effects of fixatives (Chart I.), the twenty-four lots of eggs which were placed in the twenty-four killing agents, at various intervals, had received a similar activation. Hence the living eggs, prior to fixation, contained about the same array of living cytasters. Therefore the wide diversity in cytaster structure found on the slides is the result of differing coagulation products of various fixatives

which differently coagulate the same phenomena. It will be noted, for example, that Type C, with clear rays not extending to the center, is entirely absent in certain fixatives but present in others in considerable numbers. Again, some of the reagents show large percentages of Types D and E having clear central rays, whereas others show only Types A and B, which are either without rays or have only vague ones. The significance of the relation between coarse clearly-fixed central rays and the presence of central bodies will be discussed later (p. 383).

In those fixatives containing glacial acetic acid (marked on Chart I. with an asterisk) there is a total of sixty-nine per cent. cytasters with distinct rays (Types C1 to E4), and thirty-one per cent. with rays absent or vague (Types A and B). On the other hand, in those without glacial acetic acid there is a total of ten per cent. with distinct rays and ninety per cent. with no rays or vague ones. The possible significance of acetic acid with reference to the chemical composition of astral rays will be discussed in a later paper. Experiments have been carried out concerning the effects of acetic acid, related acids, and acetates, at various hydrogen ion concentrations, as well as similar studies of other fixing agents.

The Effects of Modifications of Environmental Factors upon Rays and Central Bodies of Cytasters (Chart II.).

In the preceding experiments the activation was constant and the fixation was varied; in the present series the fixative is constant and the environmental factors of activation are varied. Chart II. presents the various modifications of activation used, *i.e.*, treatment with both weak and strong hypertonic sea water, each for forty, sixty and eighty minutes, to study the effects of differences in osmotic pressure; treatment with butyric acid solution followed by exposure to hypertonic sea water, for ten, twenty, and thirty minutes, to study the effects of modifications of the butyric-hypertonic activation technique; modifications of temperature, at two degree intervals between 20° C. and 28° C., the range within which cytasters occur when using an optimum activation, to study temperature effects; treatment with one per cent. and five per cent. ether, each for three, fifteen and

EXPERIMENT NAME AND NUMBER		ACTIVATION DATA: ETHER — PERCENTAGE OF ETHER ADDED TO SEA WATER	ETHER — LENGTH OF TREATMENT WITH ETHER AND SEA WATER MIXTURE	BUTYRIC ACID — LENGTH OF TREATMENT WITH 2cc N/10 BUTYRIC ACID PLUS 50cc SEA WATER (FOLLOWED BY 20 min. INTERVAL IN SEA WATER BEFORE HYPERTONIC TREATMENT)	HYPERTONIC SEA WATER — NUMBER OF c.c OF 2.5 N NaCl ADDED TO 50cc SEA WATER WHEN MAKING HYPERTONIC SEA WATER	HYPERTONIC SEA WATER — LENGTH OF TREATMENT WITH HYPERTONIC SEA WATER	TEMPERATURE C.	PERCENTAGE OF CYTASTER EGGS 1 hr. AFTER ACTIVATION	PERCENTAGE OF CYTOLIZED EGGS 1 hr. AFTER ACTIVATION
MODIFICATIONS OF WEAK HYPERTONIC TREATMENT	IA	No Ether Treatment		No Butyric Treatment	5cc.	40 min.	17	5	0
	IB	"	"	"	"	60 min.	"	14	5
	IC	"	"	"	"	80 min.	"	16	10
MODIFICATIONS OF STRONG HYPERTONIC TREATMENT	IIA	No Ether Treatment		No Butyric Treatment	12cc.	40 min.	17	7	22
	IIB	"	"	"	"	60 min	"	28	55
	IIC	"	"	"	"	80 min	"	35	52
MODIFICATIONS OF BUTYRIC HYPERTONIC TREATMENT	IIIA	No Ether Treatment		35 sec.	5c.c.	10 min.	17	12	0
	IIIB	"	"	"	"	20 min.	"	20	0
	IIIC	"	"	"	"	30 min.	"	60	0
MODIFICATIONS OF TEMPERATURE	IVA	No Ether Treatment		35sec.	5 c.c.	17 min.	20	0	0
	IVB	"	"	"	"	"	22	1	0
	IVC	"	"	"	"	"	24	45	0
	IVD	"	"	"	"	"	26	.1	33
	IVE	"	"	"	"	"	28	0	85
MODIFICATIONS OF 1% ETHER TREATMENT	VA	1%	3 min.	33 sec.	5c.c.	30 min.	18	3	0
	VB	"	15 min.	"	"	"	"	5	10
	VC	"	35 min.	"	"	"	"	6	14
MODIFICATIONS OF 5% ETHER TREATMENT	VIA	5%	3 min.	33 sec.	5 c.c.	30 min.	18	10	30
	VIB	"	15 min.	"	"	"	"	.1	40
	VIC	"	35 min.	"	"	"	"	0	83

CHART II. THE EFFECTS OF MODIFICATIONS OF ENVIRONMEN-

Activation: modified as listed. *Fixative:* all experiments fixed with saturated corrosive sublimate plus 2.5 per cent. glacial acetic acid. *Times of fixation:* as listed. *Percentages of types of cytasters:* each count for each experiment, at each of the three intervals of fixation, based on about 100 cytasters chosen at random from a number of eggs.

PERCENTAGES OF TYPES OF CYTASTERS AND CENTRAL BODIES

[FIXED WITH SUBLIMATE ACETIC 2.5%]

	½ HOUR AFTER ACTIVATION				1 HOUR AFTER ACTIVATION				2 HOURS AFTER ACTIVATION			
	NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT. BODY	DISTINCT RAYS TO CENTER NO CENT. BODY	DISTINCT RAYS TO CENTER GRANULAR CENT. BODY	NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT. BODY	DISTINCT RAYS TO CENTER NO CENT. BODY	DISTINCT RAYS TO CENTER GRANULAR CENT. BODY	NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT. BODY	DISTINCT RAYS TO CENTER NO CENT. BODY	DISTINCT RAYS TO CENTER GRANULAR CENT. BODY
	A	B	D	E	A	B	D	E	A	B	D	E
I A		90	10			10	65	25		12	72	16
I B		80	20			13	74	13	[No Experiment]			
I C		31	46	23		50	29	21	19	50	19	12
II A	[No Experiment]					60	37	3	25	69		6
II B	[No Experiment]					19	60	21	90	10		
II C	[No Experiment]					7	73	20	86	14		
III A	No Cytasters					12	38	50		24	66	10
III B	No Cytasters					17	39	44	[No Experiment]			
III C		1	56	43		8	71	21	[No Experiment]			
IV A	No Cytasters				No Cytasters				No Cytasters			
IV B			34	66			75	25		50	50	
IV C		45	55			45	25	30		87	13	
IV D		40	60			22	78			80	20	
IV E	No Cytasters				No Cytasters				No Cytasters			
V A	17	63	15	5		20	60	20		14	26	60
V B			95	5		8	17	75		60	18	22
V C	11	33	11	45		27	21	52		24	46	30
VI A		10	15	75		60	30	10		15	45	40
VI B	20	60	20		50		20	30	29	29	40	2
VI C	No Cytasters				No Cytasters				No Cytasters			

TAL FACTORS UPON RAYS AND CENTRAL BODIES OF CYTASTERS.

Illustrations: show only the central regions, cf. Fig. 2, p. 390. *Result:* when a fixative is used capable of clearly fixing rays, central bodies are present only when the modifications of the environmental factors of activation produce well-formed cytasters with distinct rays reaching the center. For further details cf. p. 375.

thirty-five minutes, preceding a butyric-hypertonic activation, to study the effect of an anæsthetic. The chart has a column showing the percentage of eggs in each experiment containing cytasters at one hour after activation. Another column shows the percentage of cytolysis. There are wide variations in both.

The eggs of all these experiments were fixed in sublimate acetic 2.5 per cent., *i.e.*, saturated solution of corrosive sublimate 97.5 per cent. plus glacial acetic acid 2.5 per cent. They were fixed at a half hour, at one hour, and at two hours after activation. Sublimate acetic fixatives (Chart I., Fixatives 9, 10, and 11) produce four types of cytasters, with few exceptions: (1) with no rays and no central body (Type A); (2) with vague rays and no central body (Type B); (3) with distinct central rays and no central body (Type D); (4) with distinct central rays and a vaguely demarked granular central body (Type E1). The greater variety of central body structure seen in such fixatives as weak Flemming or Bouin (Chart I., Fixatives 3 and 14) are not found in fixation with sublimate acetic.

Eggs were sectioned and stained as described above (p. 372). The percentages of cytaster types shown for each experiment, at each of the three intervals after activation when eggs were fixed, are each based on a study of about 100 cytasters and their central bodies selected at random from a number of eggs. In certain experiments, however, where cytasters are found in only one per cent. of the eggs, or less (Chart II., Experiments IV. B, IV. D and VI. B), the number of cytasters studied was about twenty-five.

In this group of experiments (Chart II.) the living eggs of the various ones contained widely differing percentages of various types of living cytasters, due to the variety of astral phenomena produced by the wide modifications of activation. These differences are visible in living cytasters by the use of a high power water immersion objective. Poorly formed cytasters appear as homogeneous structureless vesicles. Although well-formed ones do not show the clean-cut thread-like rays seen in fixed material, they clearly do show a radiate configuration. These eggs with their differing cytasters were all placed in the same fixative (sublimate acetic 2.5 per cent.), in contrast to the preceding

experiments (Chart I.) where eggs with similar cytasters were placed in different fixatives. The results of both groups of experiments confirm each other. *Using a sublimate acetic 2.5 per cent. fixation, central bodies are present only when the various modifications of activation produce well-formed cytasters with distinct rays extending to the center.* This relationship holds true whether eggs having cytasters number 0.1 per cent. (Chart II., Experiments IV. D, VI. B) or 60 per cent. (III. C); whether the cytasters arise early (III. C, etc.) or late (III. A, III. B); whether there is no cytolysis (IV. A, etc.) or 33 per cent. cytolysis (IV. D, etc.); whether the cytasters are small (5 μ) and numerous, or large (25 μ) and few; whether they are located in the egg centrally or peripherally.

The meaning of the differences in percentages of types of cytasters at one half hour, one hour, and two hours after activation, will be discussed in the next section.

The Effects of Various Intervals in the History of Cytasters upon Rays and Central Bodies (Chart III.).

In the first group of experiments environmental factors of activation are constant and the fixative is the variable; in the second group the environmental factors are the variable and the fixative is constant. In the present group both fixative and environmental factors are constant and the variable is different intervals of time during the history of the cytaster.

Chart III. presents the percentages of classes of cytasters found at frequent intervals from fifteen minutes to two hours after activation, in the case of three typical fixatives where central bodies occur, *i.e.*, weak Flemming, sublimate acetic 5 per cent., and weak picro-acetic. The counts for each interval are based on the study of about 100 cytasters and their central bodies chosen at random from a number of eggs. This is the same material from which the data for Chart I. were obtained. The sectioning and staining technique were previously described (p. 372).

It will be observed that rays first appear at about fifteen minutes after activation, remaining vague for some minutes. Clear rays are not usually found until twenty minutes after

FIXATIVE	INTERVALS AFTER ACTIVATION WHEN COUNTS WERE MADE OF CYTASTERS AND CENTRAL BODIES	PERCENTAGES OF TYPES OF CYTASTERS AND CENTRAL BODIES								
		NO RAYS NO CENTRUM A	VAGUE RAYS NO CENTRUM B	DISTINCT RAYS NOT EXTEND. TO CENTER: NO CENTRUM BY CENTRAL AREA STAINED SAME AS RAY AREA C1	DISTINCT RAYS NOT EXTEND. TO CENTER NO CENTRUM BY CENTRAL AREA STAINED LIGHTER THAN RAY AREA C2	DISTINCT RAYS EXTENDING TO CENTER NO CENTRUM D	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY VAGUELY DEMARKED GRANULAR E1	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED GRANULAR E2	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED CORPUSCULAR E3	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED RETICULAR E4
FLEMMING WEAK	15 min.		100							
	17.5 "			100						
	20 "			25		55		20		
	22.5 "			20		80				
	25 "				50	50				
	27.5 "					100				
	30 "		45	10	35	10				
	35 "			10	15	35	30		10	
	40 "			28		28	29		10	5
	45 "			6		50	17		6	17
	50 "		40	30		20		10		
	55 "		12	47		40		1		
	1 hr.		5	25		35	25	10		
	1 hr.-15 min.			50		50				
	1 " -30 "			34	16	34	16			
	1 " -45 "		17	16	17	50				
	2 hrs.		66			34				
SUBLIMATE ACETIC 5%	15 min.	34	66							
	17.5 "	70	30							
	20 "	50	50							
	22.5 "	35				34	33			
	25 "	2	10			68	20			
	27.5 "		33			34	33			
	30 "		33			34	33			
	35 "		90				10			
	40 "	45	45			5	5			
	45 "	50	50							
	50 "	100								
	55 "	100								
	1 hr.	[No Experiment]								
	1 hr.-15 min.	60	40							
	1 " -30 "	55	45							
	1 " -45 "	70	30							
	2 hrs.	50	50							
PICRO-ACETIC WEAK	15 min.	[No Experiment]								
	17.5 "	100								
	20 "	[No Experiment]								
	22.5 "	25		30		45				
	25 "		14		44	26	14			
	27.5 "			5		95				
	30 "			20	25	5	5	45		
	35 "		25	15	15		5	40		2
	40 "		50				16	34		
	45 "							100		
	50 "		12	31		19		38		
	55 "		40	30		20	10			
	1 hr.		40	30		20	10			
	1 hr.-15 min.		40	20		20		20		
	1 " -30 "		13	25	6	50			6	
	1 " -45 "		23	10		35	12	12	4	4
	2 hrs.		50	50						

CHART III. THE EFFECTS OF VARIOUS INTERVALS IN THE HISTORY OF CYTASTERS UPON RAYS AND CENTRAL BODIES.

Activation: same in all experiments; a slight over exposure of the optimum butyric-hypertonic technique, cf. footnote 4, p. 372; material same as that reported on Chart I, pp. 370–371. *Fixatives:* as listed. *Times of fixation:* as listed. *Percentages of types of cytasters:* each count for each interval of fixation, based on

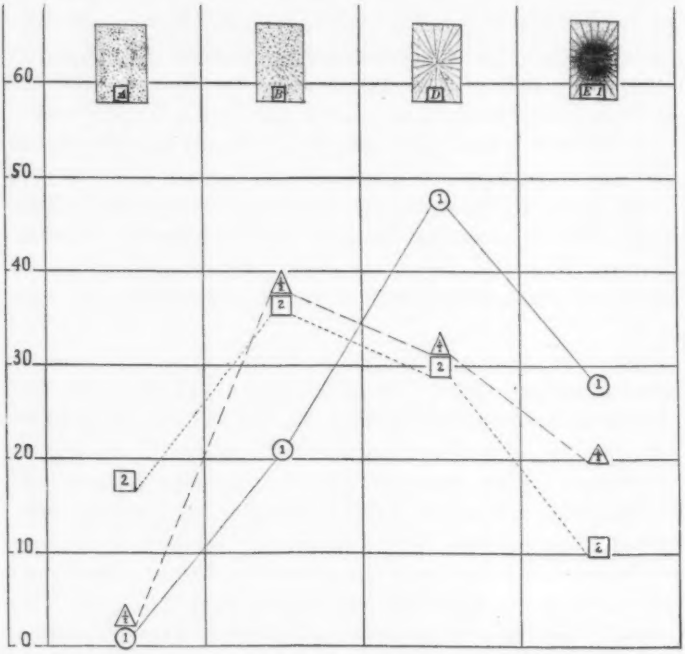
activation. Central bodies do not appear until rays have become clear, and they are never present unless the rays are distinct and reach the center. An hour or two after activation there is a tendency for the asters to become vague. When the rays become indistinct the central bodies disappear. This confirms the foregoing conclusion. *Central bodies are present only when distinct rays extend to the center of the cytaster. Cytasters pass through a formative period of vague rays when central bodies never occur; only after the rays have become clear and reach the center do central bodies appear; if the rays fade in the later history of the aster the central bodies disappear.* Thus clearly correlated with the appearance and disappearance of distinct central rays is the appearance and disappearance of central bodies. This relationship is somewhat modified by the effects of various fixatives (Chart III.) as to the percentages of types, and as to the exact time schedule, but the story is essentially the same in all.

The three fixatives reported in Chart III. are capable of showing distinctly fixed rays. The vague rays of the early and later periods of the astral history are not due to poor fixation. They are caused by the actual condition of the living rays at the time of fixation, since the same fixatives show distinct rays at the mid-period of the astral cycle. The central body phenomena shown in these three fixatives are the same as all those fixatives of Chart I. where rays are clearly fixed. Where rays are not clearly fixed there are no central bodies.

Similar results are shown in the experiments where activation is modified, Chart II. The eggs were fixed with sublimate acetic 2.5 per cent., at one half hour, at one hour, and at two hours after activation. It will be noted that in most of the experiments the highest percentage of vague (or absent) rays usually occurs at a half hour and at two hours after activation,

about 100 cytasters chosen at random from a number of eggs. *Illustrations:* show only the central regions, cf. Fig. 2, p. 390. *Result:* when an activation is used capable of producing well-formed cytasters, and a fixative is used capable of clearly fixing rays, if cytasters are studied at close intervals after activation, it is found that rays are at first vague and there are no central bodies; they appear only after rays have become clear and reach the center; they disappear when rays fade in the later history. For further details cf. p. 379.

whereas the peak of clear rays is usually at one hour. Since central bodies occur only when rays are clear and reach the center, they too show their optimum numbers at one hour, and are less numerous at one half and at two hours after activation. These relationships are shown in Graph 1. The percentages of



GRAPH 1. The effects of various intervals in the history of cytasters upon rays and central bodies.

This graph is derived from the data of Chart II., pp. 376–377. It shows the percentages of the various types of cytasters present at ½ hr., 1 hr., and 2 hrs., after activation, when all the detailed experiments concerning the effects of modified activation are averaged together. A triangle is the symbol for ½ hr. after activation, a circle for 1 hr., and a square for 2 hrs. Only cytaster types A, B, D and E1 occur, since the fixative used was sublimate acetic 2.5 per cent. The graph shows that asters with vague rays (or none) usually occur in maximum numbers at ½ hr. and at 2 hrs. after activation, whereas clear-rayed ones are most numerous at 1 hr. Also, the percentages of asters with central bodies follow the same sequence, indicating that the presence of central bodies is correlated with the presence of clear rays. For further details cf. p. 381.

each of the four types of cytasters (Types A, B, D and E1), at one half hour, one hour and two hours after activation, are in each case the total of all the detailed percentages of the various experiments for that type for that interval, shown on Chart II. A few of the individual experiments do not coincide with the average behavior, but such discrepancies are accounted for by the considerable differences in astral phenomena produced by the wide variations of activation. Despite wide modifications of osmotic pressure, temperature, butyric-hypertonic treatment, and ether treatment, producing different percentages of cytaster types, different percentages of eggs containing cytasters, different percentages of cytolyzed eggs, and different sizes and numbers of cytasters, the relationship between clear central rays and the presence of central bodies holds good.

Discussion.

The Origin of Central Bodies in Cytasters of Echinarachnius parma.

The previously described experiments prove that when eggs have been similarly activated and therefore contain similar types and percentages of cytasters, if they are fixed with a variety of killing agents, central bodies are present only when the fixative coagulates the cytaster in such a manner that rays are distinctly fixed and extend to the center (Chart I.). When, on the other hand, a fixative capable of clearly fixing rays is kept constant, but the various environmental factors used in activation are modified, the same relationship holds good, and central bodies are present only when those environmental factors produce well-formed cytasters with clear central rays (Chart II.). Finally, when an optimum activation is used capable of producing well-formed cytasters, and they are fixed by a killing agent capable of fixing rays distinctly, if the cytasters are studied at close intervals after activation, it is found that they pass through an early vague-rayed condition when central bodies are never present; only after the rays become clear and reach the center do central bodies appear; and if the rays later fade, central bodies disappear. This is strong evidence that central bodies occur only if distinct rays reach the center of the cytaster (Chart III.).

Figure 1, *a*, *b*, and *c*, shows the appearance of three serial sections (each 5 μ) of a cytaster, Type E1, that was about 15 μ in diameter. Figure 1, *d*, is a schematic reconstruction of the

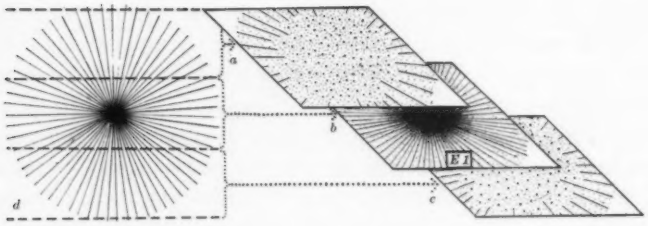


FIG. 1. The structural relations of rays and central bodies shown by a comparison of the serial sections of a cytaster with the reconstructed aster.

The serial sections (*a*, *b* and *c*), which were cut at 5 μ, are drawn from those of a cytaster having a vaguely demarked granular central body (Type E1). A schematic reconstruction of the entire aster (*d*) is shown at the left. In the top and bottom sections (*a* and *c*) rays are visible in side view in the peripheral area only, whereas in the central area they are seen in cross section as dots. In the middle section (*b*) many of the rays are visible in side view throughout their entire length, and the central portion also contains the closely crowded inner ends of the rays that extend above and below. These converge at the midpoint, and this when coagulated constitutes a central configuration, *i. e.*, the central body.

entire aster. It is obvious that the top and bottom sections (Fig. 1, *a* and *c*) present a side view of rays in only the peripheral area, and that in their central regions the rays are seen in cross section as scattered dots. It is equally apparent that the middle section (Fig. 1, *b*) presents a side view of many of the rays throughout their entire length from the edge of the cytaster to its center, and that the center also contains the inner ends of those rays extending above and below which appear in cross section as closely crowded dots.

The following hypothesis is therefore proposed as the probable explanation of the so-called central bodies seen at the center of fixed cytasters. The rays converge at the mid-point of a cytaster. When they are coagulated by fixation their closely crowded inner ends are sufficient to explain a dark configuration at the center. Since cytasters differ as to number and coarseness of rays, there is a variety of coagulation products at the mid-

points; they may be granular, corpuscular or reticular. *This study has conclusively proved that central bodies are de novo structures produced by the cytaster only after it has formed clear rays reaching the center. There is strong evidence for the further conclusion that these structures are produced only by coagulated cytasters and that they are nothing but the fixed closely crowded inner ends of well formed rays, and that they have no existence as individualized structures in the living cytaster.*

This explanation of central bodies in cytasters is supported by the fact that the type of cytaster with clear rays reaching to the center which nevertheless is without a central body (Type D), usually has rays that are more delicate than those of cytasters with central bodies (Types E1, E2, E3 and E4). Also if rays extend to the center but are vague, there are no central bodies (Type B). Therefore, only coarse well-formed rays form central bodies.

That central bodies are nothing but the coagulated bases of rays finds further support from the fact that not only must the rays be well formed, but they must also extend to the center. That type of cytaster with coarse distinct rays that do not reach the center (Type C) is of special significance in this connection. Were it not for this type it might be legitimately concluded that central bodies are individualized structures with a chemical nature similar to that of the rays; hence when rays are poorly and vaguely fixed, central bodies fail to be coagulated and appear to be absent; only when rays are well fixed are central bodies shown. The existence, therefore, of large numbers of cytasters with well-fixed distinct rays in the peripheral region that do not reach the center, disproves this possible interpretation since such cytasters never contain central bodies. If the assumption is correct that fixatives which fix central bodies are ones that fix rays, then central bodies should be present in this type of cytaster with well-fixed peripheral rays.

Almost conclusive evidence supporting the hypothesis that central bodies of cytasters are nothing but the coagulated inner ends of well-formed rays is found in the history of cytasters (Chart III.), where the appearance and disappearance of clear central rays is invariably accompanied by the appearance and disappearance of central bodies.

If, in the light of the above evidence, it is still claimed that central bodies of cytasters have an autonomous independent existence beyond the limits of microscopic vision, becoming visible only at certain times, then it must be assumed further that they always attain visibility simultaneously with the formation of distinct rays reaching the center, and again become submicroscopic whenever the rays fade; that they never attain visibility if clear rays are formed that fail to extend to the center or if vague rays arise that do reach the center. Were the central bodies of cytasters independent units having existence apart from the rays, their presence would not be so invariably dependent upon clear rays that reach the center. The wide fluctuations in structure and behavior of both central bodies and the surrounding rays caused by various fixatives, by modifications of environmental factors, and by different periods in the astral history, would not show the invariable correlation that they do. There would be at least some faint indications of central body behavior independent of clear rays reaching the center.

Although Morgan ('96, '99, '00) and Wilson ('01) did not reach such a conclusion, their numerous detailed observations of the behavior of central bodies and cytasters in *Sphærechinus*, *Arbacia* and *Toxopneustes* are the same as those of the present study. They note that cytasters are formed by the accumulation of a substance that only later assumes a radiate structure (Morgan, '96, p. 356; '99, p. 465, etc.; Wilson, '01, p. 558); that central bodies are not present at first and make their appearance only after the aster has passed through its early history (Morgan, '99, pp. 470 and 477; Wilson, '01, p. 558); that they are most apt to be present in well-formed cytasters (Morgan, '99, p. 477) whereas poorly developed ones do not contain central bodies (Wilson, '01, p. 560); that they are not present in all cytasters and show a considerable variety of structure (Morgan, '96, '99; Wilson, '01; numerous references); and that cytasters frequently fade out in their later history (Morgan, '99, p. 464; Wilson, '01, p. 554). As a result of these facts Morgan ('99, p. 477) tentatively proposed as a possibility, the conclusion established by the present study: "Whether the

centers first form and the fibers (rays) arrange themselves around them, or whether the centers are the result of the focusing of the first formed rays at a central point is difficult to determine, for both centers and rays appear at about the same time. All things considered I am inclined to adopt the latter alternative."

The plates of Morgan ('96, '99) and Wilson ('01), as well as those of other investigators illustrating echinoderm cytasters (Herlant, '19; Tharaldsen, '26, etc.), show a diverse array of cytasters and central bodies, most of which are similar to those found in the present study of *Echinarachnius*. *Toxopneustes* (Wilson, '01), however, shows certain differences. Professor Wilson kindly permitted an examination of some of his slides, and it is clear that *Toxopneustes* cytasters contain a high percentage of a central body type somewhat like E2 of the present study, but it is more compact and very much smaller. In some cases it is granular; in others it is homogeneous and is identical in appearance with a typical period-like centriole (Wilson, '01, plates XV. and XVI.). Such differences, however, due to different species, are probably of no more significance that those caused by different fixatives or differing environmental conditions. Any variations in central body structure of cytasters, no matter what the cause, are meaningless, provided the conclusion of the present study is true, that such central bodies are nothing but various coagulation products of the inner ends of well-formed central rays, having no existence as individualized structures in the living cytaster. All previous investigators of echinoderm cytasters, from Morgan ('96) and Wilson ('01) to Fry ('25*b*) and Tharaldsen ('26), have assumed that the central bodies seen in fixed cytasters are structures having an actual existence in the living egg. The present study of *Echinarachnius* proves that in this species, at least, they are but the coagulated focal point of rays. It is probable that this is true of central bodies in cytasters generally, but proof of this awaits the application of a similar method of study to cytasters of other species.

The Method of Study and its Significance for Cytological Research.

If a cell component is studied in a fixed condition it is of course necessary to check such observations with a study of living

material if this is possible. The study of living cells stained with vital dyes may yield information concerning the structure of a component when alive, to use as a basis of comparison for its appearance when coagulated. It is further necessary, if possible, to secure data concerning its chemical composition. Some components such as central bodies, are so small, however, that they cannot be seen in the living cell. Observations of such structures can be made only after the material has been coagulated and sectioned. When such is the case the greatest caution must be used in interpreting the results, keeping in mind the fact that a component when coagulated may present a very different appearance from the same component when alive.

The present study shows that various fixatives cause such a diversity of structure and behavior of central bodies, that to draw conclusions from observations made by the use of one or two killing agents might easily lead to erroneous results. A description of their structure based on fixation with Bouin's fluid would not harmonize with the results produced by sublimate acetic, and would be equally different from conclusions based on phenomena seen in material fixed with strong Flemming's fluid (Chart I.). Not only are the types and percentages of cytasters and their central bodies modified by the fixative, but the time schedule of events during the astral history is also changed. Clear rays and central bodies disappear at forty minutes after activation when eggs are fixed with sublimate acetic 5 per cent., whereas in weak Flemming's solution and in weak picro-acetic they are present until about an hour and a half after activation (Chart III.). Since the eggs were similarly activated, these time differences in the astral history are caused by the fixatives. The varying effects of different killing agents are also shown by the different percentages of cytolysis they cause, the data for which are not shown on Chart I. In the different sets, cytolysis varies from none to about fifteen per cent. at forty-five minutes after activation, and shows a much wider variation at later periods, depending upon the fixative used. Of all the factors of the environment capable of modifying the structure of a component, certainly no one of them can effect more radical changes than the killing agent. Hence if a component is studied only

in a coagulated condition it is essential to know the modifications produced not only by one or two fixatives, but by a variety of typical ones. Only when these are known is there any hope of arriving at a conception of what is a "normal" condition, and even then it must be accepted with the greatest caution.

Not only is it important to know the effects of different fixatives, but it is equally important that in the case of each fixative, accurate counts be made of all the variations of a component produced, omitting none. All the coagulation products must be considered in arriving at a result and the percentages of each should be known. Had the method of study in the present investigation been one of random search, selecting certain types as "normal" and dismissing others as "artifacts," and had all the variations in cytaster and central body structure not been studied quantitatively, it is doubtful if the real relationship between clear central rays and the presence of the central body would have been apparent. Had that type with clear peripheral rays not reaching the center and having no central body (Type C), and the type with clear central rays without a central body (Type D), as well as vague rayed asters (Type B), been passed by as due to poor fixation, and had attention been centered only on those with central body structure, the real relationship between clear central rays and central bodies would probably not have been established. It cannot be said that any of the types are more or less important than the others, or that any are "normal" or "abnormal," in arriving at the conclusion.

That cytaster with clear peripheral rays not reaching the center, in which the central area is homogeneous and stained the same as the ray area (Type C1) occasionally contains a minute deeply staining granule at its mid-point (Fig. 2). This satisfies all the requirements of one of the a priori conceptions of a "normal" central body structure, *i.e.*, one having a periodlike centriole, surrounded by a homogeneous zone, the centrosome, about which is the ray area. In fact, the present investigation began on this assumption, resulting in confusion due to the inability to explain the other types which were abundant in well-formed asters. That the central granule of this cytaster

(Fig. 2) is not a centriole, and that this aster is without significance for an understanding of central bodies in cytasters is shown by the following facts. (1) These granules are found only

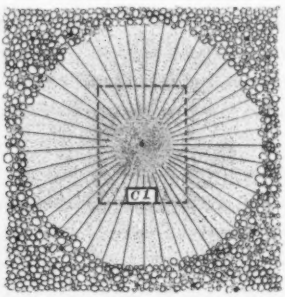


FIG. 2. Cytaster with distinct rays in the peripheral region only (type C1) containing a cytoplasmic granule at its midpoint.

This cytaster strikingly simulates a "typical" central body structure, one having a period-like centriole, surrounded by a structureless zone called the centrosome, about which is the ray area. Such a cytaster is without significance, however, concerning central body phenomena in cytasters. It occurs in but 0.5 per cent. of all cytasters. The granule is identical in appearance with those scattered through the cytoplasm. Two or three or more granules occur in such asters as frequently as does a single one.

The area within the dotted lines indicates that small portion of the midsections of cytasters shown in the chart illustrations, cf. p. 373.

in those cytasters where the rays do not reach the center (Type C1) and only in very few of them. In fact this cytaster with a centriole-like granule occurs in but 0.5 per cent. of all cytasters. Its relative scarcity was not appreciated until accurate counts were made of all the types. Until such a procedure is followed an observer can center attention upon a certain condition as typical and largely pass by other forms, and just because attention is centered on it, it may seem to be far more abundant than it actually is. Whether it occurs with a rarity that is significant can only be revealed by comparative counts. (2) The presence of a centriole-like granule in any one cytaster has no correlation with its presence or absence in other cytasters of the same egg. It is not a condition of central body structure difficult to fix, otherwise it would appear more frequently in the wide variety of fixatives used, and when present in one cytaster of an egg it

would tend to be present also in others. (3) It is important to note that such granules occur in pairs and groups of three or more as frequently as they occur singly. Wilson ('01, p. 558) observed irregular numbers of centriole-like granules, especially in the early history of cytasters. He also notes (p. 560) that in well-formed ones they may be double. (4) Most important of all is the fact that they are identical in size and appearance with the darkly-stained granules that are abundant in the cytoplasm generally. Wilson ('01, p. 557) carefully notes that granules, sometimes occurring at the centers of cytasters, are similar to those scattered through the cytoplasm. In view of the preceding points, it is clear that in *Echinarachnius* cytasters such granules that simulate centrioles are actually cytoplasmic granules that happened to be at the center of an area where an aster formed; they remain there only if the differentiation of rays does not extend to the center (Type C); they are not found at the mid-point of cytasters with clear rays reaching the center (Type D) but these contain only the much larger central bodies of a very different character previously described and illustrated (Types E1 to E4). These centriole-like granules are crowded out of cytasters if the differentiation of clear rays extends to the center, by the same forces that eliminate all relatively large particles from between well-differentiated rays in asters generally.

Morgan ('99, p. 475) notes the effects of the several fixatives he used upon the variety of central body structure seen on the slides. As a result of this he raised doubts that have been confirmed by the present work: "How large a part does the reagent play? How much is artificial and how much actually represents what is actually present in the living egg? As to nucleus and chromosomes, all series show the same results. As to cytasters, and especially as to central bodies, the door is open to skepticism." *The present investigation is a further warning of the necessity of distrusting the coagulation products of certain cellular components such as central bodies, chondriosomes, Golgi bodies, etc.; of realizing that the phenomena seen on slides may be radically different from the living condition. On the other hand, it shows with equal clearness that results may be dependable, if the various typical coagulation products are known, if all the*

types produced are studied quantitatively, and if they are all taken into consideration in arriving at a conclusion.

No profitable comparisons can be made between central bodies of cytasters and those of nuclear asters in *Echinarachnius* until the latter have been studied in a similar manner. The central bodies of the nuclear asters on the slides of the present experiments with artificially activated eggs, as well as those of cytasters that secondarily established connection with chromatin, have not been studied in detail, awaiting a controlled and quantitative study of central bodies in the nuclear asters of fertilized eggs. This is now in progress; a report of it will be presented in another paper together with a study of central bodies in fertilized eggs of *Arbacia* and *Asterias*.

Central bodies of nuclear asters in general show a great diversity of structure and behavior. In some forms they are described as autogenic, in others they appear to arise de novo; in some they seem to give rise to the aster, in others they appear only after the aster has been formed; in some they persist throughout the cell history, in others they disappear at certain phases of the cell cycle. In the light of the present study, this diversity of behavior, not to mention the variety of structure and function in the non-mitotic rôles of central bodies, makes it the more necessary to approach their future study in a controlled and quantitative manner. It is possible that if the present method is applied to the study of central bodies generally, that certain of the previous observations may be found to be based upon a single type arbitrarily selected as "normal" when using but one fixative, without knowing its quantitative relationships with respect to other types present on the same slides, and without knowing the differences caused by other fixatives. Whether or not such is the case awaits further study.

RÉSUMÉ.

1. Some cellular components, such as chromosomes, arise by the growth and division of preëxisting bodies of the same kind, maintaining genetic continuity as individualized structures from cell to cell. They, therefore, have a self-perpetuating origin. In contrast to this, other components, such as secretory droplets,

have a de novo origin, since the synthetic processes forming new units occur without reference to preformed bodies of the same kind.

2. Central bodies (a term used in its broad sense without reference to various detailed modifications) are of special interest to this problem of protoplasmic differentiation, since there is evidence that in some cases their origin is de novo, while in others it is self-perpetuating.

3. Are central bodies of cytasters in artificially activated eggs of *Echinarachnius parma*, de novo structures produced by the aster, or are they self-perpetuating bodies, that are usually submicroscopic, becoming visible only at certain times and giving rise to the aster?

4. One series of experiments proves that when eggs have been similarly activated and therefore contain similar types and percentages of cytasters, if they are fixed with a variety (twenty-four) of killing agents, central bodies are present only when the fixative coagulates the cytaster in such a manner that rays are distinctly fixed and extend to the center.

5. A second series confirms the same result. When a single fixative capable of clearly fixing rays is used, but there are modifications of the various environmental factors of activation, such as temperature, osmotic pressure, etc., central bodies are present only when those environmental factors produce well-formed cytasters with clear central rays.

6. A third series also supports the preceding conclusion. When an optimum activation is used capable of producing well-formed cytasters, and they are fixed by a killing agent capable of showing distinct rays, if cytasters are studied at close intervals after activation, it is found that they pass through an early vague-rayed condition when central bodies are never present; only after the rays become clear and central do central bodies appear; and if the rays later fade, which frequently occurs, the central bodies disappear.

7. The cytaster, therefore, produces the central body only after it has formed clear rays reaching the center, no matter what are the modifications caused by fixatives, or environmental factors, or intervals of time in the astral history. Thus the

central body has a de novo origin. It is probable, moreover, that only a coagulated aster produces a central body and that it has no existence as an individualized body in the living cytaster. The mid-point of the aster contains the crowded converging inner ends of the rays. These when coagulated form the so-called central bodies seen at the centers of cytasters in fixed material. This hypothesis is supported by the following facts: central bodies are most abundant when rays are coarse, and are usually absent when rays are delicate, and always absent when rays are vague; there are many cytasters with coarse rays in the peripheral portion which do not reach the center, and these never contain central bodies; clearly correlated with the appearance and disappearance of clear central rays during the history of cytasters, is the appearance and disappearance of central bodies.

8. The investigation is another illustration of the need of a controlled and quantitative method in cytological study. When a cellular component is too small to be studied in the living condition, and must be studied only after coagulation, it is necessary not only to control the environmental factors, and to study it at frequent intervals during periods of significant change, but it is also necessary to know its various coagulation products formed by different typical fixatives. It is equally essential to know the exact percentages of all these variations, without exception, that they may all be taken into consideration quantitatively when arriving at a conclusion. To arbitrarily select a certain type as "normal" because it happens to coincide with a certain a priori idea, and to disregard the other types as "artifacts" produces uncertain results.

9. No profitable comparison of central body phenomena in cytasters of *Echinarachnius* can be made with those of its nuclear asters until the latter have been studied in a similar controlled and quantitative manner, a report of which will be presented in another paper.

10. Glacial acetic acid was present in some of the fixatives used and absent in others. Of all types of cytasters having clear rays (whether with or without central bodies), eighty-five per cent. are found in the presence of acetic acid. The possible

significance of acetic acid with reference to the chemical composition of astral rays is under further investigation.

LITERATURE CITED.

Boveri, T.

'01 Zellenstudien IV; Über die Natur der Centrosomen. Jen. Zeitschrift, Bd. 25.

Fry, H. J.

'25a Asters in Artificial Parthenogenesis. I. The Origin of the Amphiaster in Eggs of *Echinarachnius parma*. Jour. Exp. Zoöl., Vol. 43.

'25b Asters in Artificial Parthenogenesis. II. Asters in Nucleated and Enucleated Eggs of *Echinarachnius parma* and the Rôle of the Chromatin. Ibid., Vol. 43.

Hindle, E.

'10 A Cytological Study of Artificial Parthenogenesis in *Strongiocentrotus purpuratus*. Arch. f. Ent.-mech., Bd. 31.

Herlant, M.

'19 Comment agit la solution hypertonique dans la parthénogénèse expérimentale (méthode de Loeb). II. Le méchanisme de la segmentation. Arch. Zoöl. Exp. et Gen., T. 58.

Just, E. E.

'19 The Fertilization Reaction in *Echinarachnius parma*. III. The Nature of the Activation of the Egg by Butyric Acid. Biol. Bull., Vol. 36.

Meves, F.

'02 Über die Frage ob, die Centrosomen Boveri's als allgemeine und dauernde Zellorgane aufzufassen sind. Verhand. der Anat. Gesellschaft Halle.

Morgan, T. H.

'96 The Production of Artificial Astrospheres. Arch. f. Ent.-mech., Bd. 3.

'99 The Action of Salt Solutions on the Unfertilized and Fertilized Eggs of *Arbacia*, etc. Ibid., Bd. 8.

'00 Further Studies on the Action of Salt Solutions and Other Agents on the Eggs of *Arbacia*. Ibid., Bd. 10.

'26 Genetics and the Physiology of Development. Amer. Naturalist, Vol. 60.

Petrunkewitsch, A.

'04 Künstliche Parthenogenese. Zool. Jahrb., Supplement 7.

Tharaldsen, C. E.

'26 The Origin and Nature of Cleavage Centers in Echinoderm Eggs. An Experimental and Cytological Study of Astral Phenomena in Starfish Eggs. Jour. Exp. Zoöl., Vol. 44.

Wassilieff, A.

'02 Über künstliche Parthenogenesis des Seeigeleies. Biol. Centbl., Bd. 22.

Wilson, E. B.

'01 Experimental Studies in Cytology. I. A Cytological Study of Artificial Parthenogenesis in Sea-urchin Eggs. Arch. f. Ent.-mech., Bd. 12.

'23 The Physical Basis of Life. Yale University Press.

'25 The Cell in Development and Heredity. The Macmillan Company.

Yatsu, N.

'09 Observations on Oökinesis in *Cerebratulus lacteus*. Jour. Morph., Vol. 20.

ACTION OF SALTS ON *FUNDULUS* EGG.

I. The Action of Na, K, and Ca Chlorides upon the Egg of *Fundulus*.

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Interest in the fundamental action of chemicals upon the egg, egg membrane, embryo, and larva of the marine fish *Fundulus* has centered largely around the work of Loeb and his associates (1–17). The results of this work seem to show that marked differences exist in reactions of the eggs and hatched embryos or larvæ to different salts. Marked degrees of salt antagonism are also pointed out. Inasmuch as the conclusions drawn from this work are based almost entirely upon the egg and hatched embryo or larva, it was thought highly desirable to recheck the observations, using eggs and embryos dissected out of the eggs, as well as hatched embryos or larvæ. By means of an operative technic devised by Doctor J. Nicholas [1] of Yale University, the details of which will be published elsewhere, it has been found possible to remove the outer egg membrane from the embryo and yolk sac without, in any apparent way, interfering with the further development of the embryo. As a matter of fact, embryos removed from the egg develop in sea water in as normal a way as those enclosed in the egg. It has thus been possible to compare the action of various salts directly upon the egg, the embryo dissected free from the egg membrane, and the larva hatched from the egg.

That Loeb conceived of a similarity in the action of salts upon the hatched embryo or larva and the embryo contained in the egg can easily be gathered from the following passages. In 1911 he says (6): ". . . if the fish is *out of the shell* the addition

[1] The writer is deeply indebted for the assistance rendered by Doctor J. Nicholas who was kind enough to carry out the operative technic involved in removing the egg membrane.

of $CaCl_2$ alone is no longer sufficient and the addition of KCl also becomes necessary." Other remarks in the same article seem also to indicate that the marked differences in the reactions to salts between the larva and embryo were not appreciated. In 1916 he says (8): "This prolongation of life through the addition of Ca is due not to an action upon the protoplasm but to a prevention of the diffusion of the NaCl into the egg, since if we take the embryo out of the egg (or use the newly hatched embryo) it is killed in 50 cc. 3 *M* NaCl + 1 cc. 10/8 *M* $CaCl_2$ inside of a few minutes." In his last paper on *Fundulus* (17) he says: "In 1905 the writer suggested as an explanation that a pure NaCl solution, if its concentration exceeded a certain limit, made the membrane of the egg more permeable, so that NaCl could diffuse into the egg, killing the embryo, while this increase in permeability was prevented by the presence of a low concentration of Ca." These and similar passages throughout his works seem to the writer to show that the marked differences in the reactions to salts between the embryo dissected out or freed from the egg membrane and the larva were not fully taken into consideration.

The present paper embodies results obtained from investigations carried out during the summer of 1927 at the Biological Laboratory, Cold Spring Harbor, on the effects of Na, K, and Ca chlorides on the eggs, embryos freed from the egg membrane, and larvæ of *Fundulus heteroclitus*.

Methods.

The eggs were stripped directly from the female fish into finger bowls containing sea water and then fertilized. They were kept at room temperature (20°–25° C.) as well as at lower temperatures (15°–18° C.). Those at the lower temperatures naturally took longer to reach a given stage in development than those kept continuously at the higher temperatures. The eggs were usually washed for varying periods in distilled water before use in order to free them of adhering salts as suggested by Loeb (13–17). This procedure, however, seems to the writer necessary to but a limited degree, since similar reactions are given by eggs washed for varying periods except when the time factor is

greatly lengthened, *i.e.*, to several days as was the case in many of Loeb's experiments (13–17).

The egg membrane was cut mid-laterally by means of fine pointed iridectomy scissors and the contained embryo gently rolled out of the shell by means of a fine probe. Eggs in as nearly as possible the same stages of development were used together with embryos dissected from similar eggs. It is of considerable importance that eggs and embryos be in the same stages of development, since marked age differences in reactions to some salts seem to exist. The length of exposure of the egg to water is also an important factor in changing the general consistency of the egg membrane. Both eggs and embryos were placed in covered Syracuse watch-glasses in 10 cc. of the solution and were constantly observed under a compound microscope during the course of the experiments. Five to ten organisms were used in a single watch-glass. It is of much importance to carry through experiments until the eggs hatch or the organisms die since in many cases the eggs live in certain solutions but upon further development and hatching the embryos quickly succumb. The embryos and eggs used ranged in developmental stages from those in which heart action was just beginning to those with fully developed circulation and ready to hatch. Newly hatched larvæ were used for comparison with the dissected-out embryos and eggs. The end-point observed and recorded in all the experiments herein reported was the time of cessation of the heart beat. Recovery of the heart beat in sea water was also noted but will be dealt with in a subsequent communication. The salts used in these experiments were c.p. NaCl, $CaCl_2$, and KCl made up in distilled water. Only the effects of normal solutions of these salts will be presented at this time since the results at this concentration are typical.

Results of Experiments.

Since in general the results of all experiments are qualitatively alike only typical experiments will be described.

N KCl.—KCl, as repeatedly pointed out by Loeb and his co-workers (1–17), acts with considerable rapidity on the egg, dissected-out embryo, and larva. In all, the heart quickly

TABLE I.

Salt.	Egg.	Embryo Freed from Membrane.	Larva.	Remarks.
N KCl.....	+ (75 min.)	++ (15 min.)	+++ (4–6 min.)	Penetrates membrane rather slowly
N $CaCl_2$.....	+ (98 min.)	+ (51 min.)	++ (30 min.)	Penetrates membrane rather slowly
N NaCl.....	young + (24 hrs.) older ○	○	+++ (10 min.)	Penetrates membrane rather slowly
Equal parts:				
N NaCl + *N* $CaCl_2$..	○	+ (12–24 hrs.)	++ (22 min.)	No penetration
N KCl + *N* $CaCl_2$...	++ (30 min.)	++ (20–25 min.)	++ (20 sec.)	Aids penetration
N NaCl + *N* KCl...	+ (73 min.)	++ (38 min.)	+++ (15 min.)	Penetrates membrane

Explanation.—Shows general conception as to toxic action of salts upon eggs, embryos and larvæ. + = relatively slow toxic action; ++ = a faster toxic action; +++ = a marked toxic action; ○ = non-toxic. Figures show average time for cessation of heart beat.

stops beating, the relative order of resistance being, larva < embryo < egg. The egg is about 4 times as resistant as the dissected-out embryo, and about 20 times as resistant as the larva. In the action of KCl upon the embryo within the egg, about three fourths of the time necessary to cause cessation of the heart beat is spent in the passing through the egg membrane (Table I.). Eggs with embryos in which the heart is just beginning to beat appear less resistant then older eggs.

N $CaCl_2$.—$CaCl_2$ is also extremely toxic for eggs, embryos, and larvæ, the relative order of resistance being larva < embryo < egg. The egg is about one half as resistant as the embryo, and about 3 times as resistant as the larva. Relatively less time is spent by the Ca in going through the egg membrane than by K, since the embryo outside of the egg is killed in less than one half of the total time required for cessation of the heart beat of the embryo within the egg membrane. No marked age differences in resistance to $CaCl_2$ seem to exist, since the organisms are killed at all embryonic stages in almost the same relative time.

N NaCl.—The action of NaCl is by far the most interesting of those studied, since the results obtained are quite different from those reported by Loeb (17). The relative resistance to NaCl is, larva < embryo < egg. This series, however, must be modified because with NaCl the question of age enters in a most amazing way. Freshly fertilized eggs, as pointed out by Loeb (2), are killed in solutions of NaCl. As the egg grows older its resistance to NaCl increases up to the time of hatching. Eggs in which the embryonic heart has just begun to beat are susceptible to NaCl, while the same embryo removed from the egg will usually live for days in the same solution. As a matter of fact, they live almost as long as it takes for the normal embryo in sea water to reach the time of hatching. The freshly hatched larvæ, however, are killed very quckly when put into the same NaCl solution (Table I.). A marked change in the resistance of the animal to NaCl thus takes place when the embryo is ready to emerge or emerges from the egg.

Equal parts N KCl + N $CaCl_2$.—In such a mixture the toxicity for the egg, embryo, and larva is about the same (Table I.).

The time taken to cause cessation of the heart beat, in the mixture however, is quite different from that required in the case of solutions of the individual salts, as is shown in Table I.

Equal parts N NaCl + N $CaCl_2$.—In this mixture the larva is killed in approximately 20 to 25 minutes. The embryo dissected from the egg membrane can survive from 6 to 24 hours while the eggs are not killed. As a matter of fact, eggs will hatch in the solution, and the hatched larva is quickly killed, showing quite conclusively that while in the egg the embryo is protected; once out, it quickly succumbs. The embryo dissected from the egg membrane is always found to be much more resistant than the larva (Table I.).

Equal parts N NaCl + N KCl.—Such a mixture is quite toxic for the egg, embryo, and larva, the relative resistance being: larva < embryo < egg. The egg is approximately 2 times as resistant as the embryo and about 5 times as resistant as the larva (Table I.).

Discussion.

The above results seem to the author to be of interest inasmuch as they definitely point out and show that any assumption as to the interior condition of the egg cannot be relied upon until satisfactorily tested and proven. Loeb (1–17), in most of his work on *Fundulus*, seems to have assumed that the resistance of the hatched embryo or larva was comparable to that of the embryo within the egg membrane. In the case of NaCl in particular this view is shown to be erroneous, and it is quite possible that further investigation will yield equally interesting results. That the egg membrane changes in consistency with age is quite apparent in the ease with which the membrane can be cut. Young fertilized eggs have membranes quite tough and turgid; eggs exposed to distilled water and at low temperatures for long periods of time have much softer and more pliable membranes. Associated with these structural changes are doubtless the marked physiological ones observed. Further proof of marked physiological changes in the egg membrane have come out of unpublished investigations recently conducted by Miss E. Yagle of this Laboratory, on the exosmosis of H_2O from the *Fundulus* egg of different ages. Loeb (6), also points out in

several instances that such changes occur—*e.g.*, ". . . since the *newly fertilized* egg is killed more rapidly by a *m*/2 solution of NaCl than it is killed by the same solution one or two days after fertilization." Young eggs, therefore, are very susceptible to NaCl while older ones are quite resistant. Young embryos, on the other hand, are much less susceptible than are very old ones. The hatched embryo or larva, however, is extremely susceptible to NaCl. The question as to the fundamental action of NaCl upon the younger eggs with hearts just beginning to beat apparently is not one of a purely chemical nature but rather of an action on the membrane in which the embryo is doubtless killed by some secondary effects, possibly osmotic. In eggs thus killed the contained embryo always appears much shrunken in size.

By means of the dissection technic in liberating the embryos from the egg it is possible to study quantitatively the relative effects of the salt upon the membrane and contained embryo and also upon the embryo free from the egg membrane. KCl, for example, seems to spend about 75 per cent. of its total time effect upon the egg membrane and approximately 15–50 per cent. on the embryo. $CaCl_2$ on the other hand, spends about 60 per cent. on the membrane and 30–40 per cent. on the embryo. NaCl must exert most of its effect on the membrane in those cases in which it is toxic since for embryos removed from the egg it is relatively non-toxic.

The site of action of the three salts Na, K, and Ca chlorides has always been of considerable physiological interest. Loeb (17), in the case of *Fundulus*, seems to have attributed the fundamental action of these salts to the membrane—the process being purely a diffusion phenomenon. In young eggs NaCl perhaps does not penetrate the membrane while in older eggs the membrane seems freely permeable, since the contained embryo is not killed in the same solutions when removed from the egg. KCl and CaCl seem to penetrate the egg membrane, the Ca entering in a relatively slightly shorter time than the K. The Na + Ca antagonism as suggested by Loeb (2) must be a membrane phenomenon since the embryo removed from the egg or the hatched larva is quickly killed in such a mixture. Combi-

nations of Na + K, however, act more like KCl alone and little if any antagonism seems to exist (Loeb, 1, 2). The combination K + Ca, on the other hand, seems to exert additive effects and to kill eggs and embryos in almost the same time.

Several additional facts noted in these experiments are of especial interest since they seem intimately concerned in any fundamental explanation of the salt effect upon the egg and embryo. The space between the egg membrane and embryo is at first very small, due to the large size of the yolk sac. As the embryo develops rather a large amount of fluid accumulates between the egg membrane and embryo. This increase in fluid perhaps has much to do in modifying the rates at which the embryo is killed while in the egg. Around the yolk sac and embryo there is also a delicate vitelline membrane, the properties of which seem of much importance in respect to the resistance of the embryo to various salts. If with a very fine pointed needle a minute hole is made in this membrane immediately ventral to the eye and the embryo, still normal, transferred to a solution of NaCl or left in a solution of NaCl which is not ordinarily toxic, it is quickly killed. It seems that this membrane is an important factor in determining the resistance of the embryo to NaCl. Further investigations are to be carried out on these points.

Summary.

(1) By means of an operative technic it is possible to remove the egg membrane from the egg of *Fundulus heteroclitus* and to compare experimentally the action of salts on the egg, the embryo freed from the membrane and the newly hatched larva.

(2) The effects of normal solutions of K, Na, and Ca chlorides upon the above are reported.

(3) The embryo, freed from the egg membrane, is quite resistant to NaCl solutions while the hatched larva is quickly killed in the same solution.

(4) The resistance of the eggs to NaCl increases with age.

(5) K and Ca chlorides kill the dissected-out embryo much more quickly than the egg, while the recently hatched larva is much more sensitive to the two salts than is the embryo.

(6) Combinations of these salts show antagonistic action.

Na + Ca mixtures are not toxic for eggs but are markedly so for the embryo freed from the egg membrane and for the newly hatched larva.

LITERATURE CITED.

1. **Loeb, J.** Archiv. f. d. ges. Physiol., 1894, 4, 530.
2. **Loeb, J.** Archiv. f. d. ges. Physiol., 1901–02, 38, 68.
3. **Loeb, J.** Archiv. f. d. ges. Physiol., 1905, 107, 252.
4. **Loeb, J.** Amer. Jour. Physiol., 1899–1900, 3, 327.
5. **Loeb, J.** Amer. Jour. Physiol., 1901–02, 6, 411.
6. **Loeb, J.** Science, 1911, 34, 653.
7. **Loeb, J.** Science, 1912, 36, 637.
8. **Loeb, J.** Science, 1916, 44, 574.
9. **Loeb, J., and Wasteneys, H.** Biochem. Zeit., 1911, 31, 450.
10. **Loeb, J., and Wasteneys, H.** Biochem. Zeit., 1911, 32, 155.
11. **Loeb, J., and Wasteneys, H.** Biochem. Zeit., 1911, 33, 489.
12. **Loeb, J., and Wasteneys, H.** Biochem. Zeit., 1912, 39, 167.
13. **Loeb, J.** J. Biol. Chem., 1915, 23, 139.
14. **Loeb, J.** J. Biol. Chem., 1916, 27, 339, 353, 363.
15. **Loeb, J.** J. Biol. Chem., 1917, 32, 147.
16. **Loeb, J., and Cattell, McK.** J. Bio. Chem., 1915, 23, 41.
17. **Loeb, J.** J. Gen. Physiol., 1922–23, 5, 231.

ON THE ABILITY OF CERTAIN MARINE INVERTEBRATES TO LIVE IN DILUTED SEA WATER.

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There are many reasons for believing that animal life originated in the ocean and has gradually spread through the ages into freshwater and land habitats (15, 21). In the past annelid worms invaded the soil, probably by a rather indirect route which led them first into freshwater and gradually out into the land (23). At the present time many animals in various parts of the earth show varying degrees of ability to live in diluted sea water (1, 3, 10, 11, 12, 21). Marine invertebrates which have been studied have an osmotic pressure in their blood which is approximately equal to that in sea water, but the mineral salts are somewhat less and the pressure is maintained by other substances, largely organic, which are present (1, 8, 9). The skins of various animals differ greatly in ability to control the exchange of chemicals between the body fluids and the surrounding medium. Adolph (1) studied the exchanges of substances through the skins of annelids and found that there was little resistance to them.

The writer felt that it would be of interest to determine the ability of representative marine annelids to live in diluted sea water, and during August 1927 made some observations in the Marine Biological Laboratory at Woods Hole. Thanks are due to Drs. J. A. Dawson and R. Bennitt who made suggestions and helped in the identification of several species. Mr. A. M. Hilton and his staff of collectors also made special efforts to secure materials.

Animals were brought in fresh from the field and placed as soon as possible in clean glass finger bowls containing 250 cc. of water. Tubicolous worms were removed from their tubes, except in the case of Hydroides, which was studied both in and out of tubes, and of Cystenides, which was left in its own tubes.

The water in all bowls was changed each morning, and oftener when it showed indications of becoming stagnant or when a worm bled. Dilutions were made with fresh water from the taps in the Marine Biological Laboratory. Sea water came from the same source. Page (13) has made a careful analysis of this water for mineral constituents.

The results of the observations are given in Table I. *Nereis virens*, *Laonice viridis*, and *Limulus polyphemus* showed a considerable degree of toleration for sea water diluted to one fourth its normal salinity. *Arabella opalina*, *Glycera dibranchiata*, and *Lepidonotus squamatus* lived for many days in one half sea water and one half fresh. Most of the worms tested lived several days in three fourths sea water. *Nereis* in higher concentrations of sea water climbed out of the dishes at intervals and was found in varying degrees of desiccation, hence some individuals probably died sooner than some of those in more dilute solutions. Every *Laonice* studied lived throughout the period of observation and was active at the end. The Hydroides in tubes did not live as long as those which were removed. This was probably due to the fouling of the water by small organisms in and on the mollusc shells to which the tubes of these worms were attached. The *Limuli* used were small individuals, less than 10 cm. long. All that were tested in solutions as low as one fourth sea water survived to the end of the observations. One individual lived 26 hours in a solution of one eighth sea water, and another lived two hours in fresh water.

Nereids have been observed in various localities to be noteworthy for their ability to endure considerable dilution of sea water (3, 7, 11, 13). In India there is a species of *Limulus* which lives in brackish water (3). Vaughn (24) found that several species of corals survived a reduction of twenty per cent. in the salinity of the sea water and he interpreted this as indicating that the ocean has been in the past less salty than now. The observations described in this paper show that many worms have similar toleration. The writer cannot see that general features of bodily structure and habitat are especially correlated with ability to survive in diluted sea water. Apparently delicate branchiate worms, like *Chætopterus*, *Diopatra*, and *Laonice*,

endure fresh water about as well as apparently tougher worms, such as *Phascolosoma* and *Lumbricillus*. The only oligochæte observed, *Lumbricillus agilis*, was not as hardy as many polychætes when placed in diluted sea water. In low salt concentrations all the animals studied showed a tendency to swell and became turgid and extended. *Laonice* perhaps showed this least; *Lepidonotus* and *Lumbricillus* perhaps most; *Amphitrite* frequently bled and died soon. The individual *Nereis* which lived and was active for twenty-one days in a medium containing only one fourth sea water became very active and soon began to shrink to its normal size when replaced in undiluted sea water.

TABLE I.

TIME IN HOURS WHICH ANIMALS LIVED IN SEA WATER AND VARIOUS DILUTIONS OF IT.

A indicates that an animal was apparently in good condition when the observations were discontinued.

Name of Animal.	No. Observed.	Sea Water.				
		1	¾	½	¼	⅛
Amphitrite ornata (Leidy)	12	65	115	7	2	
Arabella opalina (Verrill)	5		302*A*	302*A*	.7	
Arenicola marina (L.)	2				2	
Chætopterus pergamentaceus Cuvier	10	403*A*	403*A*	4	1	
Cirratulus grandis Verrill	4	475*A*	475*A*	8	3	
Clymanella torquata (Leidy)	13	487*A*	487*A*	38	.3	
Cystenides gouldii Verrill	3		35	256*A*	4	
Diopatra cuprea (Bosc)	17	280*A*	478*A*	30	.2	
Glycera dibranchiata Ehlers	9	498*A*	498*A*	498*A*	.7	.4
Harmothoe imbricata (L.)	7	228*A*	228*A*	4	.5	
Hydroides hexagonus Bosc	12	260*A*	260*A*	105	.5	
Laonice viridis (Verrill)	8	230*A*	230*A*	230*A*	230*A*	
Lepidonotus squamatus (L.)	13	474*A*	474*A*	379*A*	.3	
Limulus polyphemus (L.)	13	191*A*	191*A*	192*A*	192*A*	40
Lumbricillus agilis Moore	5	8	100	130	.3	
Maldane urceolata (Leidy)	1			8		
Nephthys bucera Ehlers	2	356	115			
Nereis virens Sars	13	236+	259*A*	500*A*	500*A*	3
Phascoloscoma gouldii (Pourtales)	4	252*A*	298	.3	.2	
Pista palmata (Verrill)	5	303*A*		35	26	

SUMMARY.

The ability of *Limulus*, *Phascolosoma*, and eighteen marine annelids to survive in various dilutions of sea water was studied. Most of the animals lived for a week or two in a mixture of three

fourths sea water and one fourth fresh; several species lived in one half sea water. *Limulus*, *Laonice*, and *Nereis* lived and were active for periods of two to three weeks in one fourth sea water, but died in weaker solutions.

BIBLIOGRAPHY.

1. **Adolph, E. F.**
'25 Some Physiological Distinctions between Freshwater and Marine Organisms. BIOL. BULL., *48*: 327–335.

2. **Andrews, F. B.**
'25 The Resistance of Marine Animals of Different Ages. Publ. Puget Sound. Biol. Station, *3*: 361–363.

3. **Annadale, N.**
'22 The Marine Element in the Fauna of the Ganges. Bijdr. Dierk. Amsterdam (Feest-num., M. Weber): 143–154.

4. **Benham, W. B.**
'01 Polychæte Worms. Cambr. Nat. Hist., *3*: 241–346.

5. **Blum, H. F.**
'22 On the Effect of Low Salinity on *Teredo navalis*. U. Cal. Publ. Zoöl., *22*: 349–368.

6. **Dambovinceanu, A., and Raplsine, L.**
'25 Sur le pH interieur de ceitains éléments du liquide cavitaire chez *Sipunculus nudus*. C. R. Soc. Biol. Paris, *93*: 1364.

7. **Dehorne, A.**
'25 Observations sur la biologie, de *Nereis diversicolor*. C. R. Acad. Sci. Paris, 180: 1441–1443.

8. **Dekhuysen, C.**
'21 Sur la semipérmeabilité biologique de parois exterieures des Sipunculides. C. R. Acad. Sci. Paris, 172: 238–241.

9. **Duval, M.**
'24 Recherches sur le milieu interieur des Invertébrés marins. Comparison entre le teneur en cholorure de sodium de ce milieu dt celle de l'eau de merexterieure. Bull. Sta. biol. Arcachion, *21*: 33–39.

10. **Duval, M.**
'25 (Same title.) Ann. Inst. Oceanographique Paris, N.S., *2*: 233–407.

11. **Ferronniere, G.**
'01 Etudes biologique sur les zones supralittorales de la Loire-Inferieure. Bull. Soc. Sci. Nat. Ouest Nantes, *11*: 1–45.

12. **Flattely, F. W., and Walton, C. L.**
'22 The Biology of the Seashore. N. Y., xvi + 336.

13. **Florentin, R.**
'99 Etudes sur la faune des mares salees de Lorraine. Ann. des Sci. Natur. (8), 10: 209–346.

14. **Fredericq, L.**
'22 Action du milieu marin sur les animaux Invertebres. Bull. Acad. Roy. Bruxelles (5), *8*: 423–426.

15. **Hesse, R.**
'20 Die Anpassung der Meerestiere an das Leben in Susswasser. Bonn, 1–6.

16. **Labbe, A.**
'22 La distribution des animaux des marais salants dans ses rapports avec la concentration en ions hydrogens. C. R. Acad. Sci., Paris, 175: 913–915.

17. **McClendon, J. F.**
'18 On Changes in the Sea and their Relations to Organisms. Pap. Dept. Mar. Biol., Carnegie Inst. Wash., *12*: 213–258.

18. **Packard, E. L.**
'18 A Quantitative Analysis of the Molluscan Fauna of San Francisco Bay. U. of Calif. Publ. Zoöl., *18*: 299–336.

19. **Page, I. H.**
'27 The Composition of Woods Hole Sea Water. BIOL. BULL., *52*: 147–160.

20. **Pearse, A. S.**
'26 Animal Ecology. N. Y., ix + 417.

21. **Pearse, A. S.**
'27 The Migration of Animals from the Ocean into Freshwater and Land Habitats. Amer Nat., *61*: 466–476.

22. **Redeke, H. C.**
'22 Zur Biologie der Niederlandischen Brackwasser typen. Bijdr. Dierk. Amsterdam (Feest-Num. M. Weber): 329–335.

23. **Thomson, J. A.**
'22 The Haunts of Life. N. Y., xv + 272.

24. **Vaughn, T. W.**
'19 Corals and the Formation of Coral Reefs. Smithsonian R. (1917): 189–276.

THE EFFECT OF THE QUANTITY OF CULTURE MEDIUM ON THE DIVISION RATE OF *OXYTRICHA*.

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The hypothesis advanced by Robertson (1923) that unicellular organisms produce an autocatalytic substance which aids in bringing about their own division, has been subjected to investigation by a number of workers who find little if any evidence supporting Robertson's view. In a recent paper Greenleaf (1926) presents the results of a series of experiments concerned with the effect of the amount of culture medium and cell proximity on the division rates of various Protozoa. From these results he is unable to find any evidence in favor of the autocatalytic effect. On the other hand he finds evidence corroborating the findings of Woodruff (1911), namely, that in small quantities of culture medium the division rate of isolated individuals is retarded. This is due, according to the author, to the accumulation of excretory products and their greater concentration in small amounts of medium. This retardation effect is lessened as the quantity of medium is increased.

These differences in findings leave the problem still open for investigation. It is the purpose of this brief article to set forth the results of some experiments made in an attempt to determine the relation of the amount of culture medium to the division rate of certain protozoa. No attempt has been made at this time to go into all the phases of the whole subject. The experiments were carried on with a species of *Oxytricha*. One of these organisms was isolated from a laboratory aquarium into ten drops of sterile .05 of one per cent. beef extract solution, on a depression slide. This was kept in a moist chamber made of a covered glass disk 7 x 4 x 4 inches. A pure line was established by reisolating every day for several days. When it was desired to start the experiments one or more slides were allowed to stand

48 hours or until there were sufficient organisms in one slide to start the desired number of cultures. All cultures of a given experiment were therefore descendants of a single individual isolated 48–72 hours before.

Oxytricha was chosen after a number of preliminary trials with various ciliates, because of its rapid division rate. At laboratory temperature there are usually two or more divisions in 24 hours and in rare cases as many as six generations have been produced in a day. The large number of individuals produced seemed to make the organism a more favorable subject for these experiments than other more slowly dividing ciliates.

The experiments consisted in comparing the division rate of sister cells in four and ten drops of .05 of one per cent. of sterile beef extract.

In order to keep the organisms in as near the same atmospheric environment as possible slides with two depressions were used. One depression contained the organism in four drops, the other the culture of ten drops. Four slides were used in the earlier experiments, but the number was later increased to ten. The pipettes for measuring the quantity of culture medium were drawn out so that one drop was about one one-hundredth cubic centimeter.

The first series of experiments was carried on at room temperature but as this fluctuated greatly it was decided that controlled temperature conditions were essential for reliable result. For temperatures above ordinary room temperature a Freas electric incubator was used and for temperatures below room temperatures a Cenco water bath was used, in which the moist chambers were sunk almost to their tops in the water and a constant temperature of 18° C. was maintained.

In all cases counts were made at the end of the 24-hour period. One or more slides were set aside to develop "seed" for the next day's experiments. Division rate was measured by the total number of organisms produced in 24 hours from an isolated individual in a given amount of culture medium.

In making the isolations care was taken to take individuals of as near the same size as was possible. In a culture divisions do not occur simultaneously and there are therefore small individuals

—the result of very recent division, large individuals almost ready to divide, and a majority of an intermediate size, probably those full grown. This latter group was selected for the experiments. All organisms used in one experiment were of the same line of protoplasm and as near the same age with respect to the last division as could be determined.

SERIES I.

Series I. was carried on at laboratory temperature which fluctuated very much from day to night, varying probably from 16° C. to 22° C. The results, however, are fairly constant.

Experiment.	4 Drops.		Experiment.	10 Drops.	
	No. of Cases.	Total No. Individuals.		No. of Cases.	Total No. Individuals.
1.....	4	28	1.....	4	27
2.....	4	10	2.....	4	8
3.....	4	13	3.....	4	13
4.....	4	22	4.....	4	15
5.....	4	24	5.....	4	14
6.....	4	20	6.....	4	16
7.....	4	27	7.....	4	36
8.....	4	45	8.....	4	20
9.....	4	15	9.....	4	14
10.....	4	15	10.....	4	16
	40	219		40	179
	Average, 5.48			Average, 4.47	

This indicates that at room temperature the individuals in four drops have a much higher division rate. The average division rate is 24.6 per cent. higher in the former than in the latter.

SERIES II.

Series II. was similar to Series I., except for the fact that the temperature was maintained at about 24° C. The division rate was therefore higher than in Series I.

Here again the four drops show a higher division rate than ten drops. The average for four drops is 12.3 per cent. higher than for ten drops.

4 Drops.			10 Drops.		
Experiment.	No. of Cases.	Total No. Individuals.	Experiment.	No. of Cases.	Total No. Individuals.
11.....	4	50	11.....	4	28
12.....	4	21	12.....	4	17
13.....	4	26	13.....	4	27
14.....	4	37	14.....	4	35
15.....	4	38	15.....	4	48
16.....	4	31	16.....	4	28
17.....	4	10	17.....	4	15
18.....	4	76	18.....	4	47
19.....	2	59	19.....	4	82
	34	348		36	327
	Average, 10.2			Average, 9.08	

Series III.

Since the difference in division rate in the two given amounts was larger in the lower (room) temperature (Series I.) it seemed advisable to compare the differences in rates at different controlled temperatures. Therefore, a series of experiments was run with individuals grown in four and in ten drops at 18° C. and at 23° C. The organisms used in any one day were from the same isolated individual. They were presumably as near the same age as it was possible to get them and all had been under identical environmental conditions.

18° C.						23° C.					
4 Drops.			10 Drops.			4 Drops.			10 Drops.		
Exp.	No. Cases.	Individ.	Exp.	No. Cases.	Individ.	Exp.	No. Cases.	Individ.	Exp.	No. Cases.	Individ.
B	10	19	B	9	15	A	7	41	A	10	45
D	9	38	D	10	32	C	10	59	C	10	77
F	10	32	F	10	31	E	10	83	E	10	80
H	10	46	H	10	36	G	10	103	G	10	82
L	10	44	L	10	34	K	10	138	K	10	142
N	10	26	N	10	24	M	10	87	N	9	60
P	9	36	P	10	29	O	10	97	O	10	76
	68	241		69	201		67	608		69	562
	Average, 3.54			Average, 2.91			Average, 9.07			Average, 8.1	

As in Series I. and II. the division rate in Series III. is higher in four drops than in ten drops. At the lower temperature the difference in the division rate in the two groups is greater than it is at the higher temperature. In the former the organisms in four drops have a higher rate by 21.64 per cent. while in the latter the four drops have a higher rate by 10.19 per cent.

In all of these experiments the division rate in four drops exceeds that in ten drops by more than ten per cent. If we consider only the amounts of culture medium and disregard temperature the results are as follows:

4 Drops.			10 Drops.		
Series.	No. of Cases.	Individ.	Series.	No. of Cases.	Individ.
I.....	40	219	I.....	40	179
II.....	34	348	II.....	36	327
III 18°....	68	241	III 18°....	69	201
23°....	67	608	23°....	69	562
	209	1,416		214	1,269
	Average, 6.77			Average, 5.93	

The division rate in four drops is considerably higher than that in ten drops—a difference of 14.17 per cent. This result is perhaps more significant than the others since there are over 200 cases considered.

The results of these experiments have a direct bearing on two important problems. The first of these is the question of the autocatalase. Robertson (1923) quite definitely showed that with the infusorian *Enchelys*, isolated in a limited amount of culture medium, there was an increasing reproductive rate during the second twenty-four hours of a culture over that of the first twenty-four hours. He also found that, within certain limits, division rate of *Enchelys* was higher in the first twenty-four hours in small quantities of culture medium than in larger quantities and that individuals isolated into one cubic centimeter or more of medium rarely survive.

In his interpretation of these and other experiments Robertson was forced to the conclusion that "the only possible inference

that can be drawn from this phenomenon is that infusoria discharge into the culture medium some substance which accelerates their own multiplication."

As further test of this theory he showed that reproduction would occur in sterile culture medium or in distilled water and cites the experiments of Peters (1921) with *Colpidium* to show that bacteria as food are not essential for reproduction of infusoria if the proper ingredients necessary for growth are put into the medium.

The second question for consideration is that concerning the effect of the excretory products on the division rate of protozoa. Woodruff (1911) has shown that the rate of division is very markedly lowered by the accumulation of excretory products. Greenleaf (1926) has taken up the problem and using several different infusorians arrived at the same conclusions as those reached by Woodruff fifteen years before. These results are in direct contradiction to those of Robertson and Greenleaf concludes that as far as the organisms with which he worked are concerned there is no evidence of the autocatalytic effect. He further concludes that infusoria tend to multiply faster in larger amounts of culture medium and that one individual in a given amount of medium multiplies faster than each does when two individuals are in the same amount. This difference is explained on the basis that two organisms would raise the concentration of the excretory products to a degree injurious to themselves more rapidly than a single individual would do.

The results of the experiments upon which this paper is based seem to favor the idea advanced by Robertson. In all series in which four and ten drops of culture medium were used the average division rate in four drops was higher than in ten drops. Since the culture medium was sterile when the organisms were introduced, the question of food does not seem to be one of prime importance in our consideration. The most plausible explanation therefore is that *Oxytricha* produces a substance which is liberated into the surrounding medium and which reacts on the infusorians to bring about cell division, and that the concentration of the substance necessary for bringing about cell division is reached in the smaller amount of culture medium before it is in the larger amounts.

The conflicting conclusions reached by various workers leave the question still unsolved. It is possible that the various results have been due to the fact that organisms with a high division rate show the effect of the autocatylitic substance more readily than do organisms which divide only once or possibly twice in twenty-four hours. Robertson used *Enchelys* which divides many times a day. Woodruff and Greenleaf used larger infusorians which have a much slower rate of division. Between these extremes *Oxytricha* has an intermediate division rate and in the experiments cited undoubtedly shows a higher average rate of division in the smaller amounts of culture medium. There seems to be no explanation of this so satisfactory as that suggested by Robertson for *Enchelys*, namely, that *Oxytricha* produces a substance which reacts on the organism to bring about division, and that the concentration of this substance is reached in sufficient quantities to bring about cell division in small quantities of culture medium before it is in greater quantities.

BIBLIOGRAPHY.

Calkins, G. N.

'26 The Biology of the Protozoa. Philadelphia. Lea and Febiger.

Greenleaf, W. E.

'26 The Influence of Volume of Culture Medium and Cell Proximity on the Rate of Reproduction of Infusoria. Jour. Exp. Zoöl., *46*.

Peters, R. A.

'21 The Substance Needed for the Growth of a Pure Culture of *Colpidium colpoda*. Journ. Physiol., *40*.

Robertson, T. B.

'23 The Chemical Basis of Growth and Senescence. Philadelphia, J. B Lippincott.

Woodruff, L. L.

'11 The Effect of Excretion Products of *Paramœcium* on the Rate of Reproduction. Jour. Exp. Zoöl., *10*.

CONJUGATION, DIVISION, AND ENCYSTMENT IN *PLEUROTRICHA LANCEOLATA*.[1]

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INTRODUCTION.

Evidence has been steadily accumulating of recent years that many closely related species differ from each other in number of chromosomes, the numbers often being multiples, or multiples of some common factor. Because of this fact and because the processes of conjugation have been thoroughly studied in only a very few hypotrichs, the present investigation was undertaken. *Oxytricha fallax* and *Pleurotricha lanceolata*, although placed in different genera, are morphologically quite similar, and it was thought that a comparison of the two species with respect to chromosome number and details of conjugation would be of interest. It has been shown before in at least one case (*Chilodon uncinatus*, MacDougall, 1925) that new species may arise de novo from old ones among the protozoa, the chief differences being in chromosome number, and it is not unlikely therefore that this often happens in nature. A study of division and encystment was undertaken as a natural corollary.

Pleurotricha lanceolata AND SIMILAR SPECIES.

Pleurotricha lanceolata was first described accurately by Stein in 1859, although two other species which he regards as the same had been described previously—*Stylonychia lanceolata* and *Keratium calvitium*, the former by Ehrenberg in 1832 and the latter by Müller. But Ehrenberg's organism had 16 to 18 cirri on the dorsal surface as well as the full complement on the

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ventral surface, and Müller failed to see the abdominal cirri, so that priority of description and name belongs to Stein.

Pleurotricha lanceolata was also described and figured by Kent (1880 to 1882), but his description is slightly different from that of Stein. In addition to the double row of marginal cirri on the left-hand side, Stein shows a very short third row in the middle region of the body which Kent omits, as well as the anterior third of the inner marginal row.

The race which I have used (Fig. 1) fits Kent's description very closely, but is only about half as large, and the inner marginal row of cirri is found only in the middle third of the body. Stein gives the size as varying between 83 and 143 μ, and Kent makes it just about twice as great. I have found the average size to be about 140 μ, the extremes being 100 to 165 μ, if exconjugants which are always exceptionally small are omitted.

Oxytricha bifaria (Stokes, 1887) also resembles *Pleurotricha lanceolata*, differing principally in having only a single marginal row of cirri extending around the body, and in having eight instead of six cirri on the anterior quarter.

There is also a considerable resemblance between *Oxytricha fallax* and the chief species under discussion. The former has five anal cirri arranged in an oblique row, the two posterior extending only slightly beyond the body margin.

Material and Methods.

The cultures were started from a single individual obtained from a mass culture of leaves and swamp water, which in turn was secured from Orient Springs, near Amherst, Massachusetts, November 5, 1925. These cultures were continued until May 1927, the medium being hay infusion, to which in some cases a small amount of peptone was added.

All preparations were fixed with Calkins' modification of Schaudinn's fluid, and stained with hemotoxylin, both the long and short methods being used.

Division.

The first indication of division is seen in the macronuclei, in each of which a nuclear cleft, or kernspalt, appears at the extreme

outer ends, and this gradually moves across the nuclei to finally disappear at the inner ends. Simultaneously, or very shortly afterward, a new adoral zone begins to form in the midregion of the body. This stage of division is shown in Fig. 2.

Very soon the micronuclei begin to enlarge and a clear space, or halo, appears about each. At about the same time a division center appears and divides, the two products remaining connected by a centrodesmose (Figs. 3 and 4). I have never been able to detect this endosome in the resting micronucleus however, and it is difficult to observe even in division. As the dividing nucleus enlarges it elongates, and the chromatin appears to be arranged in very fine strands, some of which seem to be in reality rows of granules (Fig. 4 *A*). At this time it stains very faintly. The chromatin next appears to become more or less concentrated at the poles (Fig. 4 *B*). As all this goes on the macronuclei change their shape, becoming almost round and the kernspalt finally passes off the inner ends (Figs. 4, 5, 6). The new adoral zone meanwhile increases in size, and new cirri may be seen developing beside the old ones of the old adoral zone (Fig. 6). In Fig. 5 the new ventral cirri may also be seen close to the adoral zones, where they first arise. As the time approaches for final separation of the daughter individuals these cirri gradually move to more nearly their normal positions (Fig. 11).

In the meantime the chromatin of the micronuclei appears to condense into discrete masses, which take the form of threads of varying length (Fig. 5 *B*). These gradually combine, until the nucleus appears to be composed of closely packed and more or less tangled threads, as in Figs. 5 *A* and 6 *B*, and finally a definite spindle appears (Fig. 6 *A*). The poles of the latter move apart and a very characteristic figure is produced which is shown in Fig. 7 *C*. Here the chromosomes appear as heavily staining Vs, the limbs of which are connected by heavy and deeply staining strands. Apparently division is really longitudinal, the two daughter chromosomes taking on a V shape and appearing connected at the ends for a while thereafter. Their final separation is shown in Fig. 7 *A*. Fig. 8 *A* shows a somewhat later stage of the metaphase.

At about this time the macronuclei begin to elongate (Fig. 8) and the remaining stages in division are passed through very rapidly, the time required being about fifteen minutes at ordinary temperatures, as compared to more than an hour for the preceding steps. The micronuclei enter the anaphase, which is quickly completed, requiring only a very few minutes, and which may be seen in Figs. 9, 9 *A*, 9 *B*, 9 *C*. The telophase is shown in Figs. 10, 11, 11 *A*, 11 *B*, and 12. The macronuclei divide at this time (Figs. 11 and 12), and the parent animal very shortly separates into two daughter individuals (Fig. 13).

Discussion.

The process of division in ciliates is so well-known that few comments are necessary, since different species differ only in minor details, and fission in *Pleurotricha lanceolata* follows the general plan. There are, however, a few points in which this species differs from others, and in other respects there are interesting resemblances.

The presence of a kernspalt is said to be common to all hypotrichous ciliates, even during the major part of the resting stage (Calkins, 1919), but I have never been able to see it in *Pleurotricha* except when the animal was about to divide, as shown by the presence of a rudimentary adoral zone. This happens even before there is any visible change in the micronuclei.

Apparently the early appearance of a new cytostome with its accompanying peristomial apparatus is characteristic of most dividing infusoria, *e.g. Uroleptus mobilis* (Calkins, 1919), *Chilodon uncinatus* (MacDougall, 1925), although there are other cases in which its development is more delayed as in *Paramœcium trichium* (Wenrich, 1926).

It seems to be generally accepted that in division there is a complete reorganization of the entire cell, extending even to the disappearance and subsequent reformation of all the organelles, including trichites in organisms which have them, undulating membranes, cirri, and even cilia. According to Wallengren (1900) the cirri of the old adoral zone in hypotrichous ciliates are gradually absorbed as new ones take their places, and I have

been able to confirm this observation for *Pleurotricha*. That this reorganization does not always extend to the old oral apparatus, however, is indicated by Wenrich (1926) who was unable to find evidence that the old cytostome disappeared and was reformed in *Paramæcium trichium*.

It is a remarkable fact that all the cirri, with the exception of the marginal ones, arise in the immediate neighborhood of the adoral zones, and indeed the first indications of their appearance are visible very shortly after the adoral zone itself appears. That this was the case in the Oxytrichidæ was noticed by Sterki (1878), and has been noted for other groups by other investigators. MacDougall (1925) found that new cilia in a dividing *Chilodon uncinatus* first appear near the new pharyngeal baskets.

The presence of a centrosome which divides as an initial step in division has until recently not been noted in ciliates. It was reported by Calkins in the first maturation division of *Uroleptus mobilis* however (Calkins, 1919), although he evidently did not see it in ordinary division, and MacDougall found it in *Chilodon uncinatus*. To this list can now be added *Paramæcium trichium* (Wenrich, 1926), and *Pleurotricha lanceolata*.

Encystment.

Encystment is a common occurrence in the life cycle of *Pleurotricha bifaria*, although what the conditions are which determine its occurrence I have been unable to discover. It may occur in old cultures, and is perhaps most frequent under those circumstances, but it also occurs in pedigreed drop cultures, which are changed every day, and in which the division rate is rapid. Of four individuals which have arisen by division from a single ancestor during the preceding twenty-four hours it is not uncommon to find one or two encysted, while the others continue to divide actively.

The account of nuclear phenomena which follows is admittedly inadequate, but it is given since no other description has been made of this stage in the life cycle of this particular species, and because the history of the cytological changes which occur in ciliates during encystment is notoriously incomplete.

The first change appearing in an individual about to encyst is

like that occurring in other infusoria under similar circumstances. The shape becomes spherical, the contractile vacuole gradually ceases to pulsate, the food vacuoles and all the organelles disappear (Fig. 14). The next step is the secretion of the cyst wall, shown in Fig. 15. It is thick, quite pliable but very tough, and without visible openings. The outside is covered with irregular, short spines. Such a cyst is shown by Stein (1859) for *Pleurotricha lanceolata*, but Cienkowsky (1855), who gives three figures of the cyst formation of *Stylonychia lanceolata*, does not show the spines. Nevertheless these two species are regarded by Stein as the same, as already stated.

Soon (probably under normal conditions in about twenty-four hours) the old macronuclei, and apparently one of the micronuclei, are bodily extruded through the cyst wall, although the opening through which they pass is never visible under any other circumstances (Fig. 16). This leaves the encysted animal in the condition shown in Fig. 17. The remaining nucleus then proceeds to divide but I am unable to say much as to the details of this division. Apparently some of the micronuclei shown in Figs. 18 and 19 are in process of division, and the process appears to be a typical mitosis. As to how a cyst in the condition of that in Fig. 19, just referred to, reaches that shown in Fig. 20 I am unable to say. Cysts are exceedingly difficult to stain and destain at this stage.

Figs. 20 and 21 show the final stages in the reorganization. As may be seen there has been a great decrease in the quantity of cytoplasm, and a corresponding decrease in chromatin. Animals at this stage may be seen swimming about within the cyst, but I have never been able to get them to leave the cyst, despite washing, prolonged observation in cultures in which the media was changed daily, and attempts to rupture the cyst wall artificially. The latter always failed because of the tough pliable nature of the wall. Both distilled water, tap water and Ringer's solution were used for washing.

Moore (1924) found that *Spathidium spathula* behaved in a similar fashion, though she was more successful in liberating encysted individuals by puncturing the cyst wall. Under ordinary circumstances few encysted animals would excyst, and

drying, which Calkins (1919) found necessary to induce the excystment of *Uroleptus*, and the addition of fresh medium, found efficacious by Moody (1912) in securing the excystation of *Spathidium*, both proved unsuccessful.

But the cytological changes which occur during the encystment of *Spathidium spathula* appear to be markedly different from those which take place in *Pleurotricha*. There is no extrusion of nuclear or cytoplasmic material from the cyst in Spathidium, but the macronuclei degenerate and finally disappear, the fragments being absorbed by the cytoplasm. The micronuclei remain practically unchanged. Shortly before redifferentiation occurs, preparatory to leaving the cyst, new macronuclear anlagen appear which strongly resemble those formed in the late stages of conjugation. It is possible that more study would show that the new macronuclei arise in a similar way in *Pleurotricha*, but I have seen no indication of it in any of my preparations.

That an extensive nuclear reorganization takes place in many infusoria during encystment has been known for a long time, but I have found no mention of the extrusion of nuclear material in any other species, although Prowazek (1899) believed that such a phenomenon occurred during conjugation. Fermor (1913) found that in *Stylonychia* there was a fusion of the two micronuclei previous to the formation of a new nuclear apparatus, and it is possible that the same thing occurs in encysted individuals of *Pleurotricha*. But in one instance I have actually observed one of the two micronuclei being extruded along with the macronuclei.

Conjugation.

During the eighteen months in which the cultures were maintained conjugation occurred but rarely, and epidemics of it were never observed. Even in mass cultures containing thousands of individuals it was usually necessary to look for an hour or more to obtain a few pairs. Because of the small number of conjugants no sections were made and all the results herein described were obtained from whole mounts, fixed with Calkins' modification of Schaudinn's fluid, stained with hematoxylin, and mounted individually. Various methods of inducing conjugation have been

suggested by investigators who have found them more or less useful with certain species, and a number of these methods were tried in this case, but with practically no success.

Conjugating individuals fuse by the adoral surfaces (Fig. 22), the entire peristome of one member of the pair disappearing completely. There seems to be no reason why it cannot function in the other conjugant but there is very little evidence that it does so, for food vacuoles become fewer in number as conjugation progresses, and exconjugants are always small. The time from fusion to final separation varies from eighteen hours to five days, but the usual duration is twenty-four hours.

Behavior of the Micronuclei.

The micronuclei normally go through three maturation divisions, only two taking part in each. The others degenerate more or less rapidly, although some may persist even after the interchange. The pronucleus usually undergoes two cleavage divisions. Of the four products one enlarges and eventually gives rise to the macronuclei of the reorganized exconjugant, one degenerates, and the remaining two form micronuclei.

The First Maturation Division.

This division requires more time than any of the others—at least eight hours—and is also strikingly different in type. The micronuclei at first show no change but soon increase in size, become surrounded by a clear space, or "halo," and stain more faintly than usual. The chromatin takes on a finely-granular appearance. Shortly an endosome, or division center, appears, and in favorable preparations two may be seen (Fig. 28 *A*, *C*), but I have never been able to detect an intradesmose connecting them, although in vegetative division and in the other divisions of conjugation it can often be seen. This division center increases in size until it becomes hemispherical and stains very heavily (Fig. 28 *C*, *D*). In the meantime spindle fibers begin to appear, and the spindle takes on the typical parachute appearance (Fig. 28 *C*, *D*, *E*). The chromatin in the expanded top of the parachute, which is at first composed of very small, dimly staining granules, condenses into larger granules which stain

more heavily and these pair to form heavily staining chromosomes of dumbbell shape. These granules are altogether too numerous to count, and in most preparations this is also true of the chromosomes, but I have made counts in a few cases which will be discussed at greater length below. The chromosomes now move to the center of the spindle and divide longitudinally (Fig. 28 *F*, *G*). Stages of the anaphase and telophase are shown in Figs. 28 *H* to *M*. The most characteristic feature of this division, aside from the dumbbell shape of the chromosomes, is the way in which they lag and the peculiar curve at the poles of the spindle.

The Second Maturation Division.

This follows rapidly on the first, and is over in much less time, with the incidental result that material showing it is difficult to obtain. It is also of very different character. The micronuclei which are to divide enter the prophase almost before the telophase of the preceding division is complete (Fig. 32 *A*). At this stage they stain faintly, and rows of granules appear which are arranged in a more or less "whorled" fashion (Fig. 32 *B*). Fig. 32 *C* represents a stage which is seldom seen, but it is presumably earlier than those shown in the two preceding figures. The nucleus next enlarges and the rows of granules become threads (Fig. 32 *D*). This may well be in reality a leptotene stage, since reduction occurs during this division. There is soon evidence of a definite spindle which is at first of a peculiar oval shape (Fig. 32 *E*, *F*). I have been unable to get preparations showing the metaphase and later stages of the anaphase, but the early anaphase is shown in Fig. 32 *F*. I have made a number of counts of the number of chromosomes concerned in this division, the average result being forty. Two stages of the telophase are shown in Fig. 32 *G* and *H*.

The Third Maturation Division.

The third division differs in type from both the preceding, and requires more time than the one just described. The micronuclei which are to divide enlarge, and become very finely granular. I am uncertain as to whether there is an intradesmose

connecting the products of the division of the intranuclear endosome, but I believe that there is. What is apparently one end of it can be seen in Fig. 36 *A*. These granules now condense to form rows, and the latter in turn become much coiled chromosomes which often appear double (Fig. 36 *B*, *C*). The metaphase is shown in Fig. 36 *D*, and is considerably like that of ordinary vegetative division. The chromosomes in both the second and third maturation divisions are rod-shaped, in contrast to the dumbbell shape which they have in the first, and the V and irregular rod shape of the cleavage divisions. The long-pointed anaphases are characteristic of the third division, and long drawn-out telophase (Figs. 36 *E* to *I*).

The interchange is a rapid process, and the wandering nuclei do not appear to differ in any respect from the stationary ones. New adoral zones appear at this time or very early during the first cleavage division (Fig. 37).

The First Cleavage Division.

The amphinucleus is shown in Fig. 40, and is apparently divided into two parts, doubtless representing the two pronuclei. The stages in the first cleavage division are shown in Figs. 41 *A* to *H*. The nucleus, which increases very much in size shortly after fusion, becomes smaller, and forms a peculiar sort of spindle (Fig. 41 *A*), in which the chromatin appears to be condensed into a heavy ribbon, twisted more or less upon itself, and quite definitely double. Chromosomes soon appear which are arranged in bouquet fashion, about a small endosome, and which at this stage seem to be, in some cases at least, in pairs (Fig. 41 *B*). They then straighten out and form a characteristic spindle which has at first two definite parts, possibly representing the two pronuclei (Fig. 41 *C*). At this time the chromosomes are definitely double, and apparently twisted about each other. The metaphase is figured in 41 *D*, and the anaphase soon follows. Apparently the chromosomes in the later stages of this division are shaped like very acute Vs. Figs. 41 *E* to *H* show the anaphase and telophase. The former is rather characteristic in its early stage because the chromosomes are so widely separated within the receding plates.

The Second Cleavage Division.

The first cleavage division which has just been described is a rapid process, but the second one is much slower. A division center appears, as in two of the three maturation divisions, and divides, the products remaining connected by an intradesmose (Fig. 46 *A*). The nucleus then becomes elliptical, and the chromatin becomes arranged in long deeply-staining strands which are at first quite regular in arrangement (Fig. 46 *B* and *C*). These elongate and become twisted (Fig. 46 *D*, *E*), some of them appearing double. The whole spindle resembles nothing so much as a tangled skein of yarn at this stage, and possibly the stage represented in Fig. 46 *D* is in reality a spireme. The strands in the following figure are definitely polarized and probably are really chromosomes. The metaphase is shown in Fig. 46 *H*. It does not differ very much from that in the first cleavage division. The anaphase is very peculiar, as can be seen from Figs. 46 *J* to *M*. At the poles of the spindle a "cap" of very deeply-staining chromatin is formed and the chromosomes lose the definite shape which they had in the metaphase, apparently coalescing into more or less irregular masses which take a very heavy stain. These are very characteristic and make the later stages of the second cleavage division differ from all the others. The strands persist for some time in the anaphase (Fig. 46 *L*, *M*).

The Occasional Third Cleavage Division.

Following the second division there may in some cases be another, but when it occurs only two of the four nuclei take part as a rule, so that exconjugants in which this third division has taken place have six nuclei, rarely eight. All the stages of this division which I have observed are like corresponding ones of the second division.

The Behavior of the Macronuclei.

The anterior of the two macronuclei elongates during the maturation divisions, and may occasionally divide by a process of mass division, but only in very rare cases. At the same time it undergoes a slow fragmentation, so that the cytoplasm usually

has a number of heavily staining masses of chromatin at this time, which often simulate degenerating micronuclei derived from previous divisions. The posterior macronucleus may elongate slightly, but I have never observed it to divide and it undergoes much less fragmentation than the other.

The chromatin fragments and degenerating micronuclei usually disappear about the time of the interchange, or soon afterward, but may in some cases persist until after the cleavage divisions.

Reorganization of the Exconjugant.

Reorganization begins before separation, and usually requires several days. One of the four nuclei resulting from the second cleavage division becomes very large and coarsely granular, and at the same time loses much of its capacity to stain with hemotoxylin. It is destined to form the macronuclei of the reorganized exconjugant and is shown in Figs. 47, 48, 49, 50, 52 and 53. This nucleus divides at the first division of the exconjugant, and then divides again without corresponding division of its possessor, thus restoring the normal macronuclear condition (Fig. 55).

But it is apparently possible for reorganization to become complete without prior division of the exconjugant, and an individual in which this has happened is shown in Fig. 54. It may be that this can happen in individuals in which there has been a third cleavage division.

The micronuclei of the reorganized conjugant are derived directly from two of the three which remain from the second cleavage division. The third persists for a time but eventually degenerates. As to what happens when there have been three cleavage divisions I am unable to say, since I have been unable to observe enough cases.

The remains of the old macronuclei persist for a day or two as more or less circular, deeply staining and vesicular masses of chromatin (Figs. 49 to 52), but they eventually disappear completely, leaving no trace (Fig. 53).

The Number of Chromosomes.

In order to determine the number of chromosomes a large number of counts were made in several different stages, par-

ticularly from an especially favorable preparation showing the early anaphase of the first maturation division, and from several preparations of the anaphase of the third maturation division.

Since the total number of chromosomes is so large in the anaphase of the first maturation division accurate counts are difficult, but ten were made as a check for the other counts described below. The mean of these ten counts was eighty-six, which would indicate that the diploid number is forty-three. The standard deviation was found to be 15.4, the coefficient of variation 17.9, and the probable error $\pm$ 3.46. There is no doubt therefore that the counts are very significant.

A few counts were made of the chromosomes in the early anaphase of the second maturation division, and the average was close to forty, again indicating therefore that this figure is close to the diploid number.

Since the most favorable preparations for chromosome counting were those of the third maturation division these were given the most study. To insure results as free from error as possible seventy counts were made from several preparations showing the anaphase of this division. A curve was constructed of these counts and the mode found to be 19. The mean was 19.62, and the probable error $\pm$ .295. The coefficient of variation was 18.6 per cent., and the standard deviation 3.66. It is therefore certain that the counts are highly significant, and there is no doubt that the haploid number of chromosomes is close to twenty, with a high degree of probability that it is exactly that number, making the diploid number forty.

Since the number of chromosomes in *Oxytricha fallax* is twenty-four (Gregory, 1923), there is therefore no obvious relation as far as number of chromosomes is concerned between this species and *Pleurotricha lanceolata*.

Discussion.

Although only a relatively small number of ciliates have been studied with reference to the phenomena of conjugation the process appears to be essentially the same in all. From his own observations Maupas (1888) divided it into eight phases—*A*, the period of preparation preceding the first meiotic division; *B*,

the first division; *C*, the second division; *D*, the third division; *E*, the interchange and fusion of the pronuclei; *F*, the first cleavage division; *G*, the second cleavage division; and *H*, the period of reorganization preceding the first fission of the exconjugant.

These phases have been shown to hold for all infusoria so far studied, including *Pleurotricha lanceolata* which is the subject of this paper, although in a few forms such as the Vorticellidæ, Ophryoscolecidæ, and *Euplotes patella*, there are one or more preliminary divisions before the meiotic divisions begin. (In the case of the Vorticellidæ this happens only in the case of the microgamete).

Ciliates as far as at present known fall into two classes according to the behavior of the micronuclei in the first phase—those which undergo a prophase like *Paramœcium*, the micronucleus being drawn out into a crescent, and those in which a parachute or candelabra-like figure is formed, to use Calkins' term. To the last group belong *Onychodromus grandis* (Maupas, 1888), *Bursaria truncatella* (Prowazek, 1899), *Didinium nasutum* (Prandtl, 1906), *Anoplophrya branchiarum* (Collin, 1909), *Uroleptus mobilis* (Calkins, 1919), *Oxytricha fallax* (Gregory, 1923) and *Chilodon uncinatus* (MacDougall, 1925). The character of the first division, already described, makes it necessary to add *Pleurotricha lanceolata* to this list.

As might perhaps be expected from the close morphological resemblance of *Oxytricha fallax* and *Pleurotricha lanceolata* the details of the conjugation process are much alike, and while they are also similar to those which occur in *Uroleptus mobilis* as described by Calkins, the resemblance is much less striking.

The formation of the parachute preliminary to the first maturation division more nearly resembles the corresponding stages in *Uroleptus* than in *Oxytricha*, but differs from both. In the latter, although the parachute fibers are focused on a single granule derived by division from the endosome just as they are in *Pleurotricha*, there is also a centrodesmose connecting the two halves of the endosome which I have never been able to observe in *Pleurotricha*. In *Oxytricha* there appears to be no endosome, and the place of the basal granule is taken by a row of granules.

The chromosomes in this division appear to be formed by the fusion of the granules into which the chromatin is divided early in the prophase, but whether the number of these granules bears any constant relation to the number of chromosomes, as Gregory believes it does in the case of *Oxytricha*, I am unable to determine. These chromosomes which at first make up the top of the parachute appear to move down the fibers until they reach the center of the spindle when they form an equatorial plate. The other pole of the spindle appears to be formed by one of the two halves of the endosome which remains in the top of the parachute, the other as already stated, forming the granule at its base. This is apparently the same as in *Uroleptus*.

The number of nuclei taking part in any of the three maturation divisions is apparently never greater than two in the case of *Pleurotricha*, although forms in which there are three micronuclei occur. Of the four products of the first division all but two degenerate, and the same thing happens after each of the other two maturation divisions. The number of nuclei taking part in each maturation division in *Oxytricha* appears to be variable, although there are always at least two. In other multinucleate ciliates which have been studied there is considerable variation in this respect. In *Bursaria* all the sixteen or more nuclei may take part in the first meiotic division (Prowazek, 1899), and in *Uroleptus* there may be two, three or four primary spindles (Calkins, 1919), but these are exceptions.

The second maturation division is apparently of different character from the first in most ciliates, and lacks the elaborate preliminaries of the latter, with the result that it requires much less time. The micronuclei in most cases do not return to the resting stage between the first and second division, although there are exceptions (*e.g.*, *Chilodon*, MacDougall, 1925). In all these respects therefore *Pleurotricha lanceolata* is typical.

In nearly all cases in which a careful study has been made reduction has been found to take place in the second meiotic division, although the first division was thought to be the reducing division in *Paramœcium caudatum* (Calkins and Cull, 1907), and in *Oxytricha fallax* (Gregory, 1923). But Dehorne (1920) regards the third division as the one in which reduction occurs in the

former form. In the case of *Pleurotricha* there is no doubt but that it occurs in the second, for the following reasons: The total number of chromosomes taking part in the first division in the beginning anaphase has been determined to be in the neighborhood of eighty, as already stated, whereas there are only half as many concerned in the second division, and the average number moving toward each pole of the third maturation spindle has been determined, as stated above, to be about twenty.

The only protozoa in which reduction is known to occur at any other time (with the two exceptions already mentioned), are *Diplocystis* (Jamieson, 1920), and *Aggregata eberthi* (Dobell, 1915). It is suggested by Dobell and Jamieson that this may be a universal occurrence among the Telosporidia.

The third maturation division and the interchange take place exactly as in *Oxytricha*, and are very much alike, even to details in the form of the spindle. Nothing is said as to the existence of a division center or intradesmose however. The dumbbell character of the chromosomes is not lost until the third division in the case of *Oxytricha*, and it is stated that the chromatin streams toward the poles in the form of granules. I find that the chromosomes are definitely rod-shaped in *Pleurotricha*, in both the second and third divisions, although the rods are heavier and shorter in the former, and may give place to rows of granules in the late anaphase stages of the latter. The dumbbell character is never regained after the first of the three maturation divisions.

The two cleavage divisions follow the usual course, again resembling closely those in *Oxytricha*, although not many details are given in Gregory's paper. The products of the second cleavage division are equal as in *Oxytricha*, but differ in this respect from the state of things in *Uroleptus mobilis*, in which one of the two nuclei derived from the first division gives rise to the macronuclei and the other to the micronuclei of the reorganized exconjugant (Calkins, 1919). I have found no other case in which the anaphase of the second cleavage division resembled that in *Pleurotricha* however, and it differs strikingly from that in *Oxytricha*.

The occasional third cleavage division is also a striking charac-

teristic, and as far as I can discover it occurs in no other hypotrich so far described, although it is admitted that the number studied is thus far small. In other forms such as *Paramœcium caudatum* (Calkins and Cull, 1907), and several of the Vorticellidæ, a third division of the amphinucleus occurs, but as a regular thing. In *Bursaria* (Prowazek, 1899), there are four divisions before differentiation occurs.

The behavior of the macronuclei in *Pleurotricha* follows the usual rule—degeneration eventually occurs, although it is not completed until after separation. But division of the macronuclei, although apparently the rule in *Oxytricha*, is very rare in *Pleurotricha*.

The time required for reorganization and the details of the process differ considerably in different cases, but the process as it occurs in *Pleurotricha* is not significantly different from the essential features of reorganization in other species. Prowazek believed that the remnants of the old macronuclei must be extruded bodily from the cell in *Stylonychia pustulata* and *Bursaria truncatella* (1899), but I have seen no evidence of this in the present instance, nor has it ever been observed by anyone else. He based his hypothesis on the fact that nucleins are indigestible.

The Physiological Effect of Conjugation.

The significance of conjugation has been much disputed, one school holding that it is the necessary result of senescence in any given race, and that by it the death of the race could be averted and rejuvenation, expressed chiefly in an acceleration of the division rate, be secured. Maupas held this view and since his time Calkins has been its leading exponent. Another group has regarded conjugation as a process useful chiefly as a means of producing variations, which are much greater and much more frequent in exconjugants than in ordinary vegetatively reproducing individuals. Jennings has been one of the chief expounders of this view.

Many experiments have been tried to settle this question and since the results have differed considerably for different species I have endeavored to find out the effect of conjugation in *Pleuro-*

tricha lanceolata, as measured by the division rate of exconjugants when compared to closely related non-conjugating lines.

The experiment consisted in isolating one hundred exconjugants, and following them daily as long as possible. Of this number 92 per cent. died without division, usually about four days after separation, and of the remaining eight only one gave any evidence whatever of rejuvenation. The others divided two or three times and then all died. This single individual gave rise to a line which divided between two and three times a day for six days, and from then until the death of the line four weeks later, the average daily division rate gradually declined until during the last two weeks it was much less than one per day.

The controls during this period averaged from one to two divisions a day, and showed no evidence of senescence for the six months for which pedigreed cultures were maintained. Both controls and exconjugants were in the same media.

It is realized that one hundred cases is scarcely enough to draw final conclusions from, and yet the percentage of deaths is so high that it does not seem likely that a greater number of cases would have given significantly different results.

Jennings (1913) in a long and comprehensive series of experiments demonstrated that with *Paramœcium* "Conjugation decreases the rate of fission, causes a great increase in variation in fission rate, brings about many abnormalities, and greatly increases the death rate," but Mast (1917), using *Didinium nasutum*, was unable to secure evidence of any such effect. He found that there was no appreciable effect on either death rate, fission rate, or increase in variation of fission rate, thus proving however that there was also no rejuvenation.

Calkins, in a long series of experiments with *Uroleptus mobilis*, has shown that in this species at least conjugation appears to have a genuine rejuvenating effect (Calkins, 1919 and 1926), and Woodruff and Spencer (1924) conclude that conjugation has a similar effect in the case of *Spathidium spathula*, although they prefer the term "high survival value" to that of "rejuvenation."

It has been shown by many investigators working with various species that as culture methods are improved the longevity of cultures without conjugation can often be indefinitely increased,

so that it can probably be safely concluded that conjugation is at least not a necessary process if environmental conditions are sufficiently favorable.

Since cultures of *Pleurotricha* have been maintained for eighteen months with very little conjugation (none in the pedigreed lines which were carried for six months), and since in the cases mentioned above none of the exconjugants gave rise to lines which continued for any length of time, although kept under identically the same conditions as controls which maintained a uniform and fairly high division rate, it can be concluded I think that conjugation is not an indispensable part of the life cycle. This conclusion is supported by Baitsell (1914) who carried on experiments of this nature with both *Pleurotricha lanceolata* and *Oxytricha fallax*.

It is nevertheless apparent that a process which is so universal among infusoria as conjugation must serve some useful purpose, and that in nature exconjugants do not always die. Why then do they die in cultures? It is impossible to give a definite answer, but it has been suggested that media which is suitable for active vegetative multiplication may not always be suitable for conjugation and exconjugants, and this may well be the explanation.

Summary.

1. *Pleurotricha lanceolata* is a hypotrichous ciliate belonging to the family Pleurotrichidæ. The species has as its chief characteristic six anal cirri, divided into two groups. The anterior of the two groups consists of four cirri—three very large ones arranged in an oblique row, and a smaller one, a little forward of the posterior end of the row. The second group includes two cirri, both of them very long and projecting well beyond the posterior end of the animal. The usual size of vegetative individuals is 140 μ.

2. The process of division does not differ particularly from that in other infusoria, except that in the very early stages an endosome appears and divides, the products remaining connected by an intradesmose. This has been described in *Paramœcium trichium* but in few if any other species. The process of division is initiated by the appearance of a rudimentary adoral zone and

a kernspalt in each of the macronuclei. The micronuclei divide by typical mesomitosis. When division of the latter is virtually complete the macronuclei follow suit, dividing amitotically, and then the cell itself divides. All organelles appear to be regenerated, the old ones being absorbed.

3. Encystment may occur at any time, and appears to bear no relation to periods of depression, to division or to conjugation. The old macronuclei are extruded bodily from the cell, and a single micronucleus remains. It is uncertain whether the other is always extruded with the macronuclei or fuses with the first micronucleus. An uncertain number of micronuclei are formed from the single remaining micronucleus, and from these the normal nuclear complex is rebuilt, the process being complete at the time the animal is ready to leave the cyst.

4. The nuclear changes which occur during conjugation are essentially the same as those described for other ciliates. There are three maturation divisions, an interchange of pronuclei, and two or rarely three cleavage divisions. The four products of the second division are at first alike, but one soon enlarges and eventually gives rise to the new macrônuclei of the reorganized exconjugant. One of the others degenerates and the other two form the new micronuclei. The old macronuclei degenerate after separation of the exconjugants. There may or may not be one division of the latter before reorganization is completed.

5. Reduction occurs in the second maturation division. The diploid number of chromosomes is forty, as nearly as can be determined, and the haploid number twenty. The chromosomes are dumbbell-shaped in the first maturation division, and rod-shaped in the second and third. They are also rod-shaped in the cleavage divisions, but the shape then is unlike that in the maturation divisions or in vegetative division.

6. Each of the divisions in conjugation differs from all the rest and from vegetative division.

7. Conjugation occurs but rarely in the race of *Pleurotricha* used, and under the conditions of culture virtually always results in the death of the conjugants a few days after separation. In only one instance out of a hundred did it result in anything like rejuvenation, and even in this case the daughter race died within a month.

8. Although the details of conjugation are much alike in *Oxytricha fallax* and *Pleurotricha lanceolata* there is no obvious relation between the number of chromosomes of these two very similar species.

Conclusion.

The cytology of conjugation and division in *Pleurotricha lanceolata* has been described in detail, together with some of the cytological phenomena of encystment. The processes of division do not differ particularly from those which are known to occur among ciliates in general, with the exception of the dividing endosome and connecting intradesmose. Certain features in the division of the micronucleus are strikingly like some which occur in the mitosis of metazoan tissue cells.

A remarkable feature of encystment is the extrusion of the old macronuclei, and perhaps one of the micronuclei, through the cyst wall. After this has happened the normal nuclear complex is rebuilt from the single micronucleus remaining. Whether the presence of the latter is regularly due to the extrusion of the other micronucleus from the cell, or to the fusion of the two original micronuclei is not altogether certain.

The cytological changes of conjugation are in general similar to those previously described in other ciliates, and especially resemble those which occur in *Oxytricha fallax*, but there are important differences in detail. Reduction is shown to occur in the second maturation division. The diploid number of chromosomes is too great to determine exactly, but is probably forty. There may be a third cleavage division in addition to the two which regularly occur, and in this respect *Pleurotricha lanceolata* differs from any other hypotrich so far described.

Conjugation appears to be not only an unnecessary part of the life cycle of this species, at least as long as environmental conditions remain favorable, but is a very dangerous event, for 92 per cent. of one hundred exconjugants died without further division, and only 1 per cent. showed any indication of an accelerated fission rate.

BIBLIOGRAPHY.

Baitsell, G. A.

'14 Experiments on the Reproduction of the Hypotrichous Infusoria. II. A Study of the So-called Life Cycle of *Oxytricha fallax* and *Pleurotricha lanceolata*. Jour. Exp. Zoöl., Vol. 13, pp. 211–234.

Calkins, G. N.

'19a *Uroleptus mobilis*, Engl. I.—History of the Nuclei during Division and Conjugation. Jour. Exp. Zoöl., Vol. 27, pp. 293–357.

'19b *Uroleptus mobilis*, Engl. II.—Renewal of Vitality through Conjugation. Jour. Exp. Zoöl., Vol. 29, pp. 121–156.

'26 Biology of the Protozoa. Philadelphia. 623 pp.

Calkins, G. N., and Cull, S. W.

'07 Conjugation of *Paramecium aurelia* (*caudatum*). Arch. f. Protistk., Vol. 10, pp. 375–415.

Cienkowsky, L.

'55 Über cystenbildung bei infusorien. Zeitschr. f. wiss. Zool., Vol. 6, pp. 301–306.

Collin, B.

'09 La conjugaison d'*Anoplophrya branchiarum* St. Arch. d. Zool. Exp. et Gen.—5th Series—T. 1, pp. 345–388.

Dehorne, A.

'20 Contribution a l'étude comparée de l'appareil nucléaire des Infusoires ciliés, des Euglenes et des Cyanophysees. Arch. Zool. Exp. et Gen., Vol. 60, pp. 47–176.

Ehrenberg.

'32 Die infusionsthierchens. S. 373, Taf. XLII.

Fermor, X.

'13 Die Bedeutung der Encystierung bei *Stylonychia pustulata* Ehr. Zool. Anz., Bd. 42, pp. 380–384.

Goodey, T.

'23 The Excystation of *Colpoda cucullus* from its Resting Cysts and the Nature and Properties of its Cyst Membranes. Proc. Roy. Soc., Vol. 86, pp. 427–439.

Gregory, Louise H.

'23 The Conjugation of *Oxytricha fallax*. Jour. Exp. Morph., Vol. 37, No. 3, pp. 555–581.

Jamieson, A. Pringle.

'20 The Chromosome Cycle in Gregarines, with Special Reference to *Diplocystis schneideri*. Quart. Jour. Mic. Sci., *64*: 2, pp. 207–266.

Jennings, H. S.

'13 The Effect of Conjugation in *Paramecium*. Jour. Exp. Zoöl., *14*: 279–391.

'20 Life, Death, Heredity and Evolution in the Protozoa. Boston. 233 pp.

Kent.

'80–'82 Manual of the Infusoria. London. 2 vols. 913 pp. 51 plates.

MacDougall, M. S.

'25 Cytological Observations on Gymnostomatous Ciliates, with a Description of the Maturation Phenomena in Diploid and Tetraploid Forms of *Chilodon uncinatus*. Quart. Jour. Mic. Sci., *69*: Pt. 3, pp. 361–384.

Mast, S. O.

'17 Conjugation and Encystment in *Didinium nasutum* with Especial Reference to their Significance. Jour. Exp. Zoöl., Vol. 23, No. 2, pp. 335–359.

Maupas, E.

'88 Recherches experimentales sur la multiplication des Infusoires ciliés. Arch. d. Zool. Exp. et Gent., Vol. 6, pp. 165–277.

'89 Le rajeunissement karyogamique chez les ciliés. Arch. d. Zool. Exp. et Gen., 2d Series, T. 7, pp. 149–517.

Moody, J. E.

'12 Observations on the Life History of Two Rare Ciliates, *Spathidium spathula* and *Actinobolus radians*. Jour. Morph., Vol. 23, pp. 349–396.

Moore, E. Lucile.

'24 Endomixis and Encystment in *Spathidium spathula*. Jour. Exp. Zoöl., Vol. 39, No. 2, pp. 317–337.

Prandtl, H.

'06 Die konjugation von *Didinium nasutum* O.F.M. Arch. f. Protist., Bd. 7, pp. 229–258.

Prowazek, S.

'99 Protozoenstudien (*Bursaria truncatella* und *Stylonychia pustulata*). Arb. a. d. Zool. Inst. Univers. Wien., Bd. 11, pp. 195–268.

Stein, F. R.

'59 Der organismus der infusionsthiere. Leipzig-Abdruck 1, 206 pp.

Sterki, V.

'78 Beitrag zur Morphologie der Oxytrichen. Zeit. f. wiss. Zool., Bd. 31 pp. 29–58.

Stokes, A. C.

'87 Ann. and Mag. Nat. Hist., Aug. 1887, pp. 110–111.

Wallengren, H.

'00 Zur Kenntnis der vergleichende morphologie der hypotrichen infusorien Bih. T, k. Svenska vet. Akad. Handl., Vol. 26, 31 pp.

Wenrich, D. H.

'26 The Structure and Division of *Paramecium trichium* Stokes. Jour. Morph. and Phys., Vol. 43, No. 1, pp. 81–102.

Woodruff, L. L., and Spencer, H.

'24 Studies on *Spathidium spathula*. II. The Significance of Conjugation. Jour. Exp. Zoöl., Vol. 39, No. 2, pp. 133–196.

EXPLANATION OF THE FIGURES.

Magnification (unless otherwise stated) × 550; spindles × 1700.

PLATE I.

Early Stages of Division.

FIG. 1. A typical vegetative individual. Occasionally individuals are found with only one micronucleus, or with three.

FIG. 2. The first stage in division. A kernspalt appears at the outer end of each macronucleus, and the beginning of a new adoral zone can be made out (a. z.).

FIG. 3. The micronuclei show an endosome, or division center, in process of division, the halves being connected by an intradesmose. The kernspalt has moved nearer the center of each macronucleus

FIG. 4. The micronuclei have become somewhat larger, and the macronuclei have become almost round. The kernspalt is almost ready to pass off the latter entirely, and the new adoral zone is more conspicuous.

FIG. 4 *A*. An elongated, faintly staining micronucleus; seen in very early division.

FIG. 4 *B*. Same as above, but a little later.

FIG. 5. The chromatin in the micronuclei has condensed into definite threads. The macronuclei have become almost completely spherical and the new adoral zone is well developed. New cirri are making their appearance in the neighborhood of both adoral zones. Enlarged figures of the micronuclei are given under 5 *A* and *B*.

FIG. 6. The anterior micronucleus, shown enlarged in Fig. 6 *A*, has formed a definite spindle.

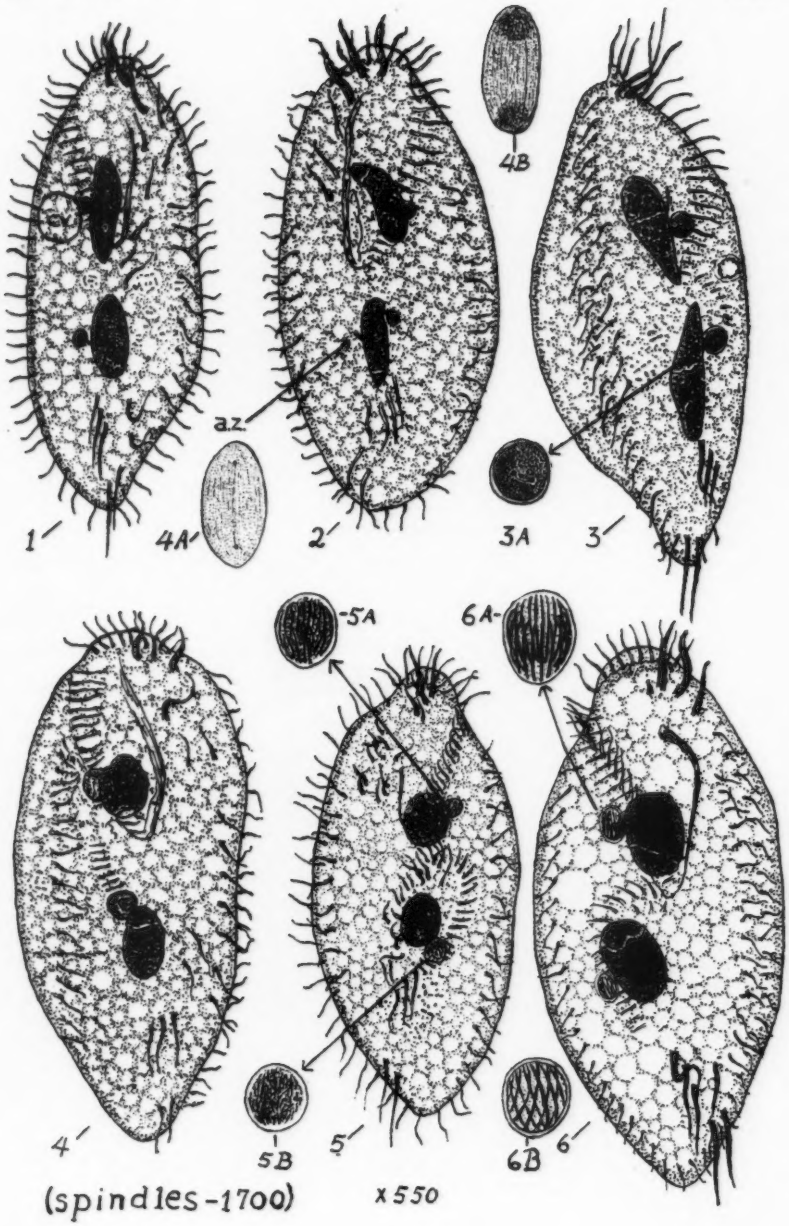

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PLATE II.

Later Stages of Division.

FIG. 7. Both micronuclei have formed spindles, which are shown enlarged in 7 *A* and 7 *B*. The cirri are larger, and the macronuclei still almost round.

FIG. 7 *C*. A spindle, magnified about 3500 times, showing the shape of the chromosomes, and the way in which they divide. The heavy strands connecting them are to be noted particularly.

FIG. 8. An individual with but one micronucleus, which is in the metaphase. The macronucleus have elongated, and the new cirri are conspicuous and large.

FIG. 9. An individual in which the micronuclei are in the anaphase.

FIG. 9 *C*. A later stage in the anaphase.

FIG. 10. A still later anaphase.

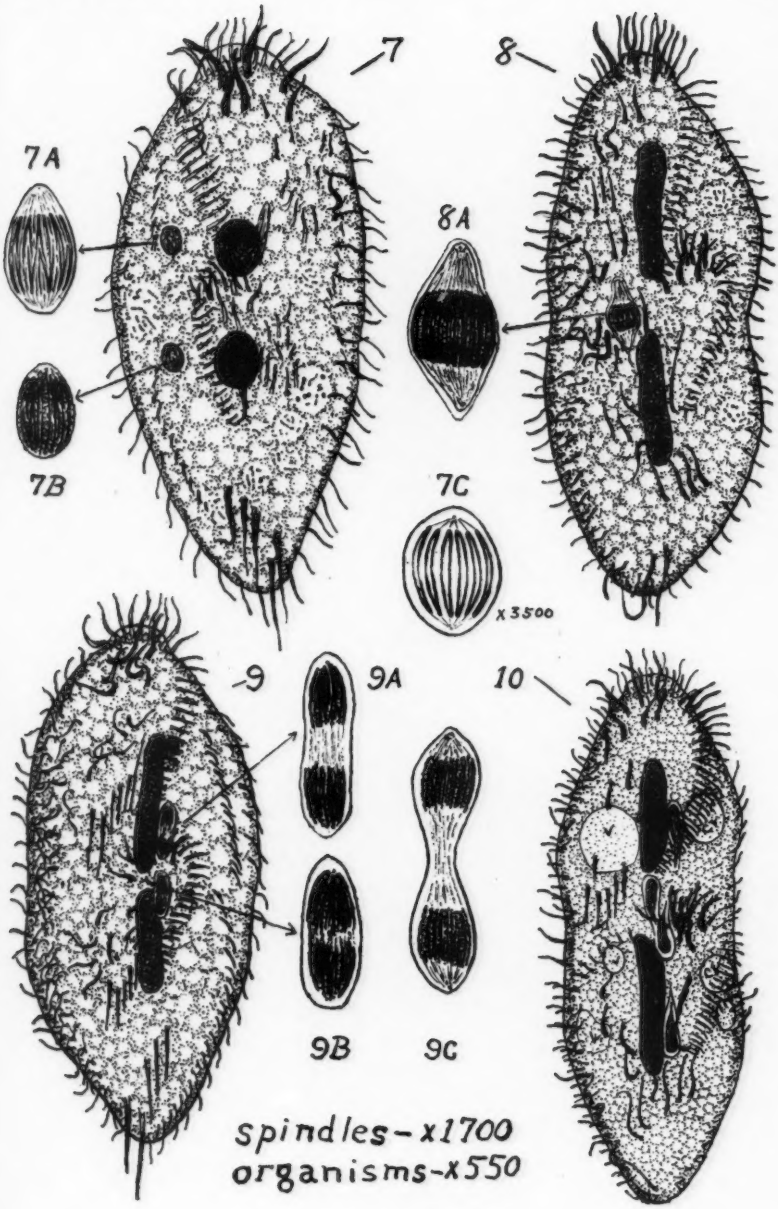
7
8
7A
8A
7B
7C
x 3500
9
9A
10
9B
9C
spindles - x1700
organisms - x550

PLATE III.

Final Stages of Division; Cysts.

FIG. 11. The macronuclei are beginning to divide, and some of the old cirri have apparently disappeared. The new ones, originally formed near the adoral zones, are approaching their normal positions.

FIG. 11 *A*. A late stage in the telophase, enlarged from Fig. 11.

FIG. 11 *B*. An earlier telophase.

FIG. 12. The final stage of division.

FIG. 13. An individual which has just divided. The shape differs somewhat from that of a typical vegetative individual, and the anal cirri are not yet quite in their normal positions.

FIG. 14. An early stage of encystment, in which the individual has rounded up, but some of the cirri still remain, and the contractile vacuole is still functional. × 750.

FIG. 15. An encysted animal, in which the cyst wall has just been secreted. × 750.

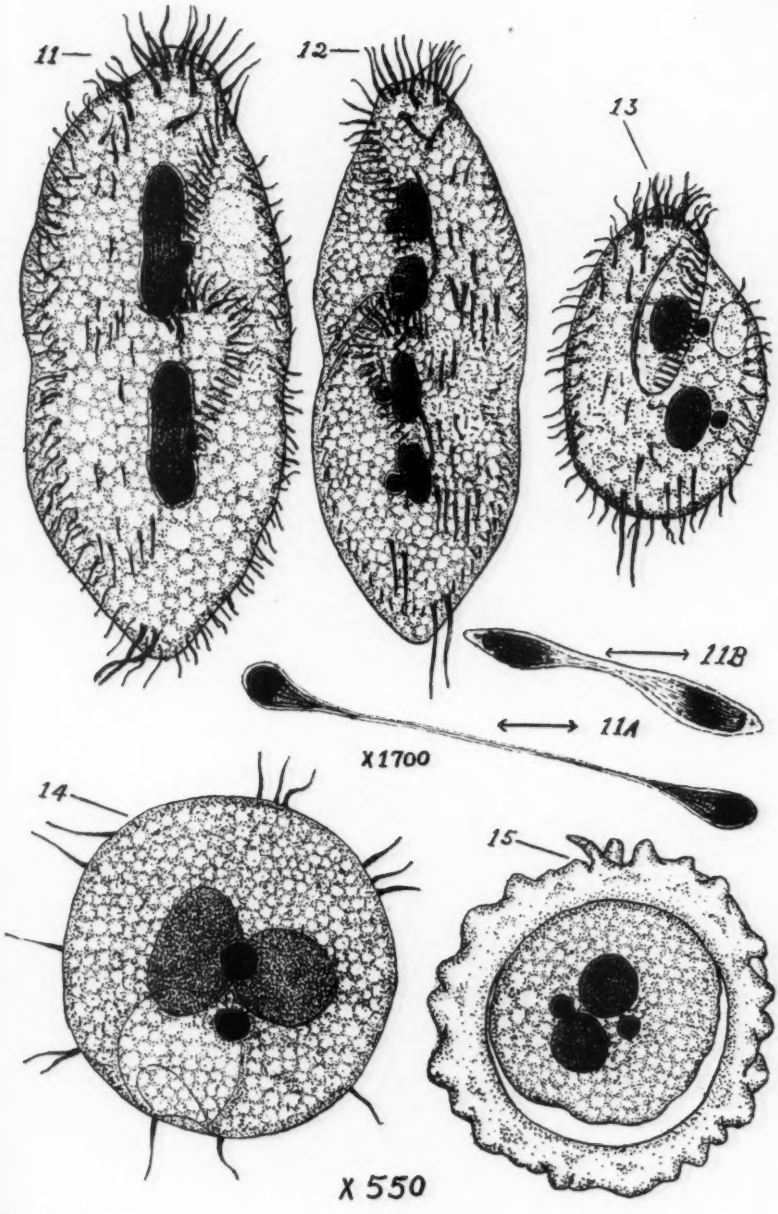

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PLATE IV.

Cysts in Various Stages.

FIG. 16. Showing the extrusion of the macronuclei and a small portion of the cytoplasm.

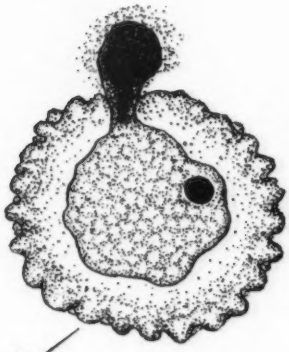
FIG. 17. An encysted animal in which only one micronucleus remains. × 750.

FIG. 18. A cyst containing three nuclei and some darkly staining material which is however not chromatin. × 750.

FIG. 19. A cyst containing seven nuclei, several of which appear to be in mitosis. × 750.

FIG. 20. A cyst in which reorganization is almost complete. × 750.

FIG. 21. Cyst containing a completely reorganized animal. × 750.



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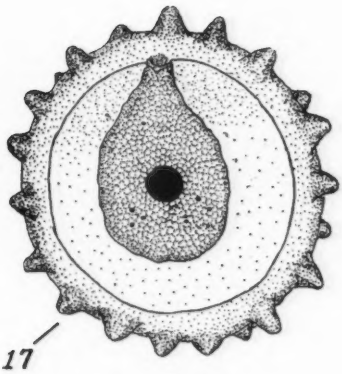

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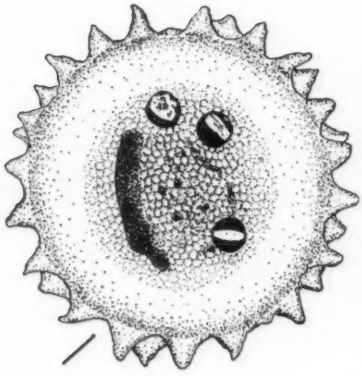

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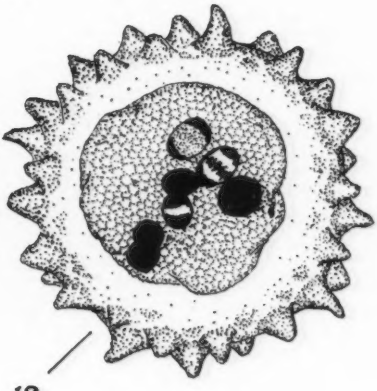

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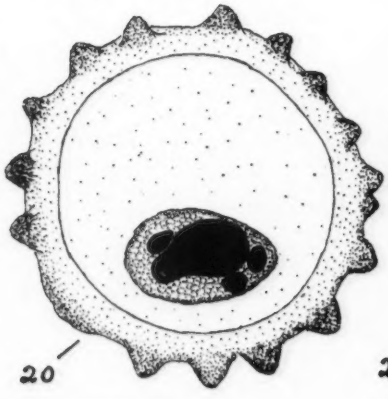

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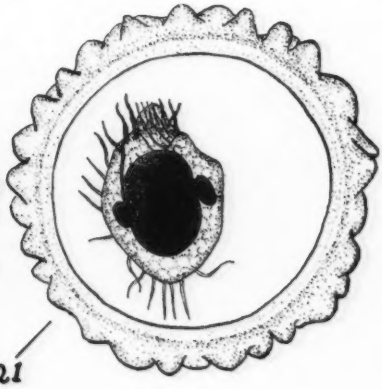


21

PLATE V.

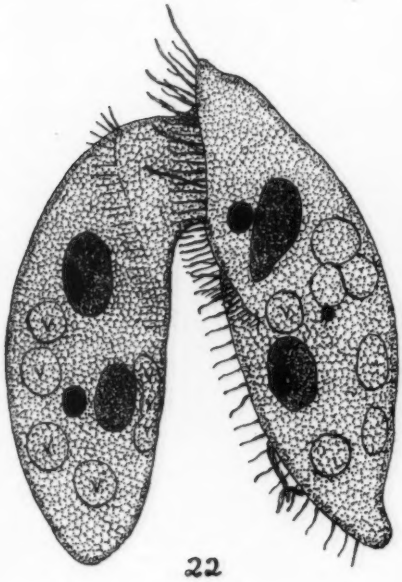
First Maturation Division.

FIG. 22. Initial stage in conjugation, showing the manner of fusion.

FIG. 23. A pair in which the micronuclei have begun to enlarge, preparatory to the first maturation division.

FIG. 24. Here the division centers have made their appearance, and in one nucleus the spindle fibers are becoming visible. Enlarged in 28 *B* and 28 *C*.

FIG. 25. Parachute stage, just prior to the formation of chromosomes. Shown enlarged in Fig. 28 *E*.



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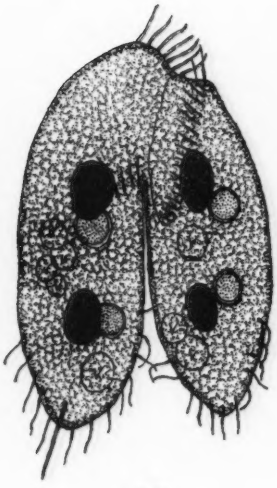

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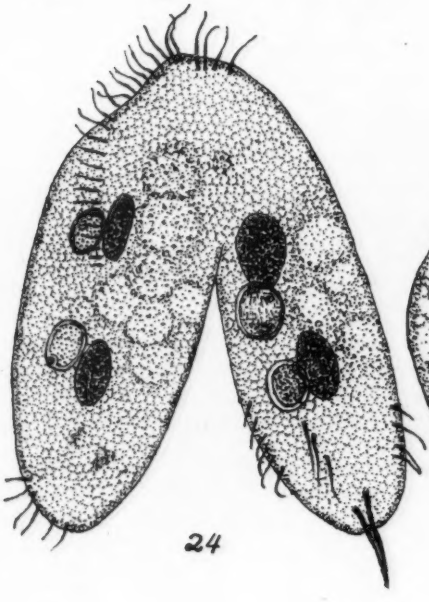

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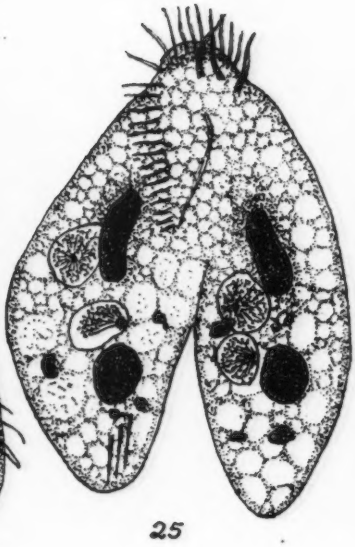


25

PLATE VI.

First Maturation Division.

FIG. 26. Here the micronuclei have entered the anaphase. Two of the spindles are shown enlarged in Figs. 28 *H* and 28 *K*.

FIG. 27. A pair in which a telophase may be seen in each member.

FIG. 28 *A–M*. Various stages of the first maturation division arranged consecutively.

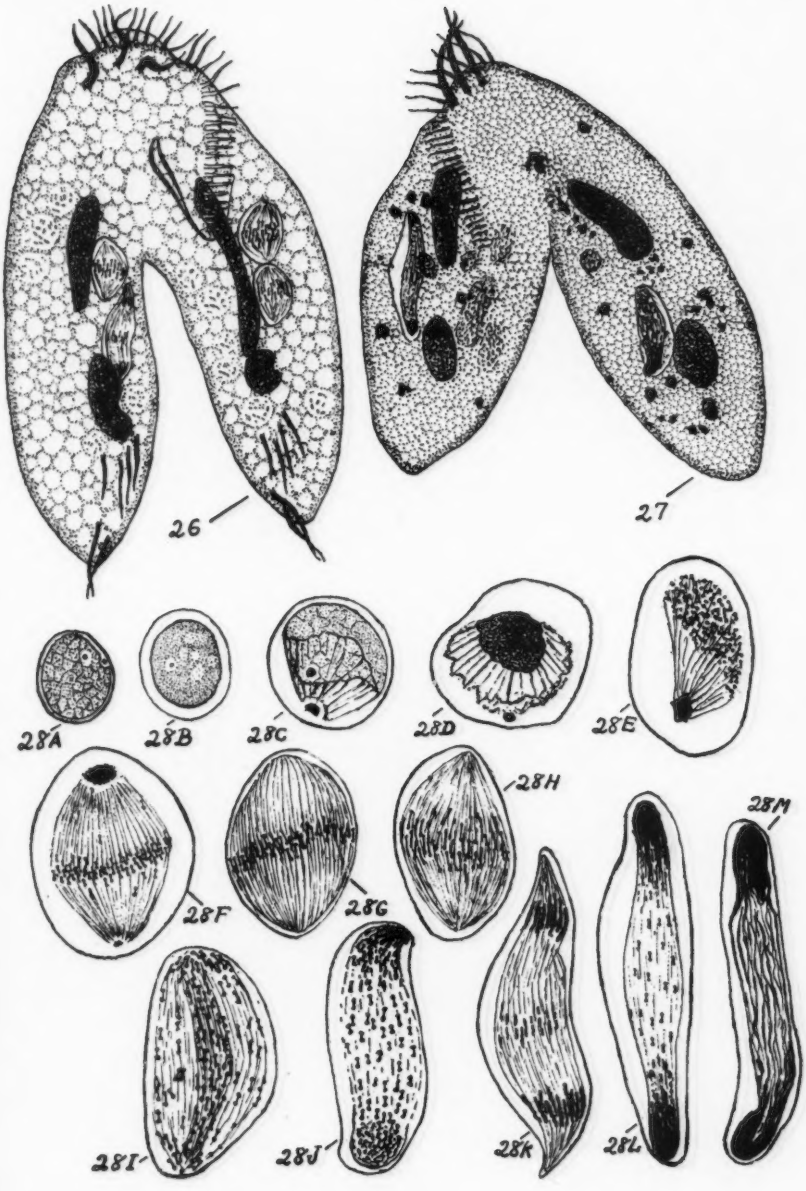

26
27
28A
28B
28C
28D
28E
28F
28G
28H
28I
28J
28K
28L
28M

PLATE VII.

Second Maturation Division.

FIG. 29. The left-hand member of the pair shows the final stage in the telophase of the second maturation division. On the right there is a prophase and an early anaphase.

FIG. 30. Each individual of this pair shows a telophase of the second maturation division, and a prophase of the third division is also to be seen in the left-hand member.

FIG. 31. Two prophases of the second maturation division are visible in the left-hand member and a prophase of the third division may be seen on the right.

FIG. 32 *A–H*. Various stages in the second maturation division. "*A*" shows both products of the first maturation division, one of which is already in the prophase of the following division. "*C*" is also a prophase, and should probably precede "*A*."

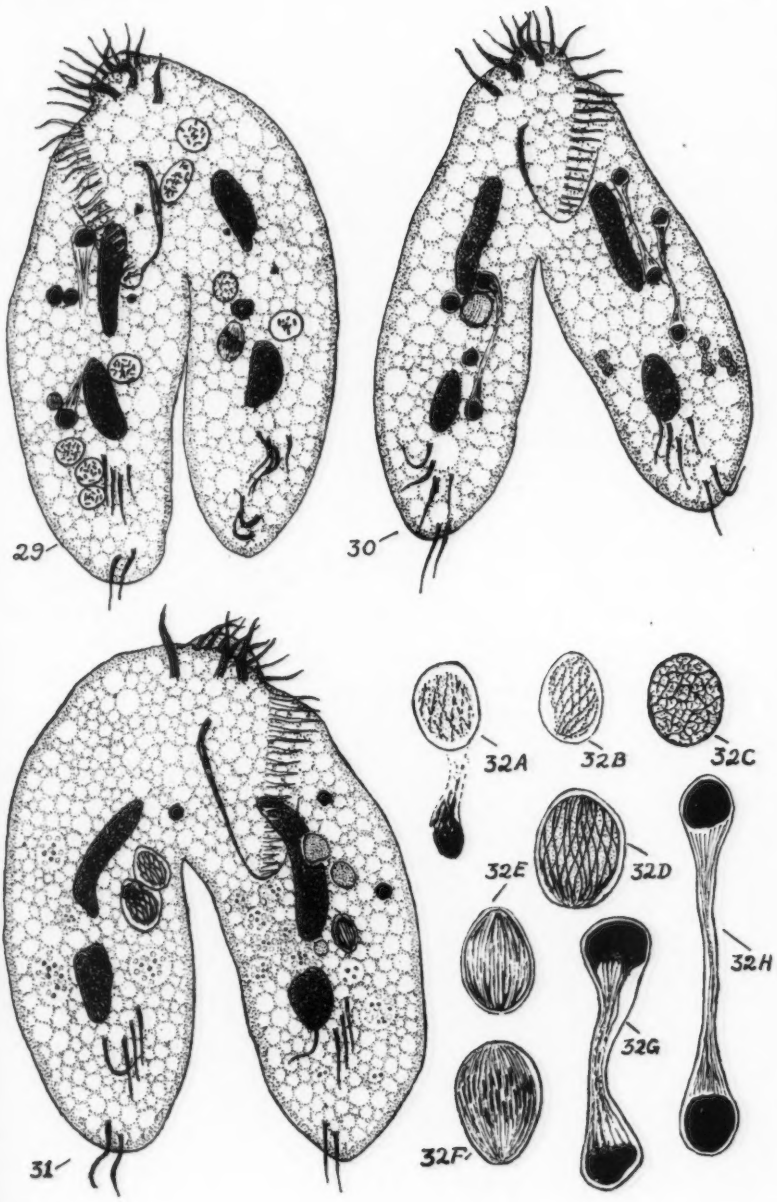

29
30
31
32A
32B
32C
32D
32E
32F
32G
32H

PLATE VIII.

Third Maturation Division.

FIG. 33. A pair undergoing the third maturation division. Two anaphases appear on the left and a prophase and anaphase on the right.

FIG. 34. Each member of the pair shows a metaphase, and in addition there is an anaphase in the left-hand member and a prophase in the one on the right.

FIG. 35. A metaphase and anaphase appear on the left, and a metaphase and telophase on the right.

FIG. 36 *A–I*. Stages of the third division, arranged consecutively.

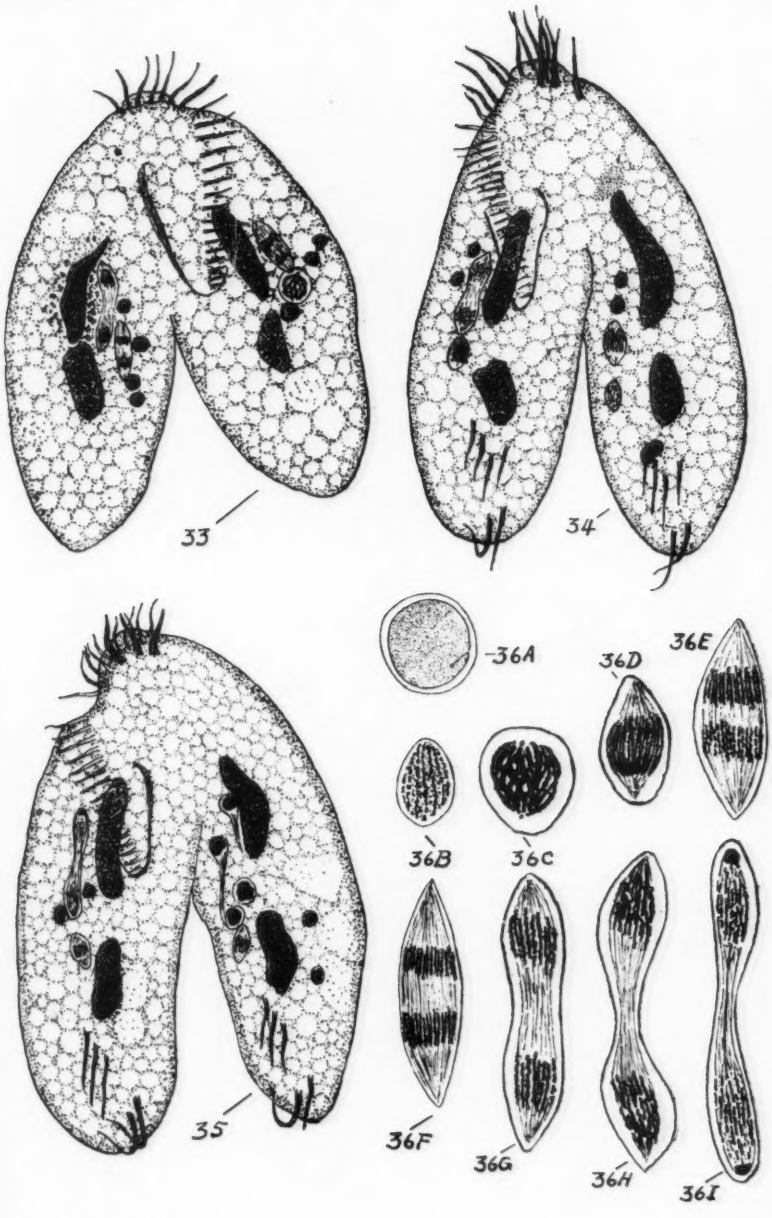

33
34
35
36A
36B
36C
36D
36E
36F
36G
36H
36I

PLATE IX.

Interchange and First Cleavage.

FIG. 37. The interchange. The wandering nuclei have already migrated but have not yet fused. Shown as large and relatively faintly staining bodies. New adoral zones are beginning to form.

FIG. 38. The left-hand member of this pair shows the fusion nucleus.

FIG. 39. In the left-hand individual the pronucleus has not yet entered the prophase of the first cleavage division, but division in the right-hand member is well advanced.

FIG. 40. The pronucleus of Fig. 38, enlarged × 1700.

FIG. 41 *A–H*. Stages of the first cleavage division, arranged consecutively.

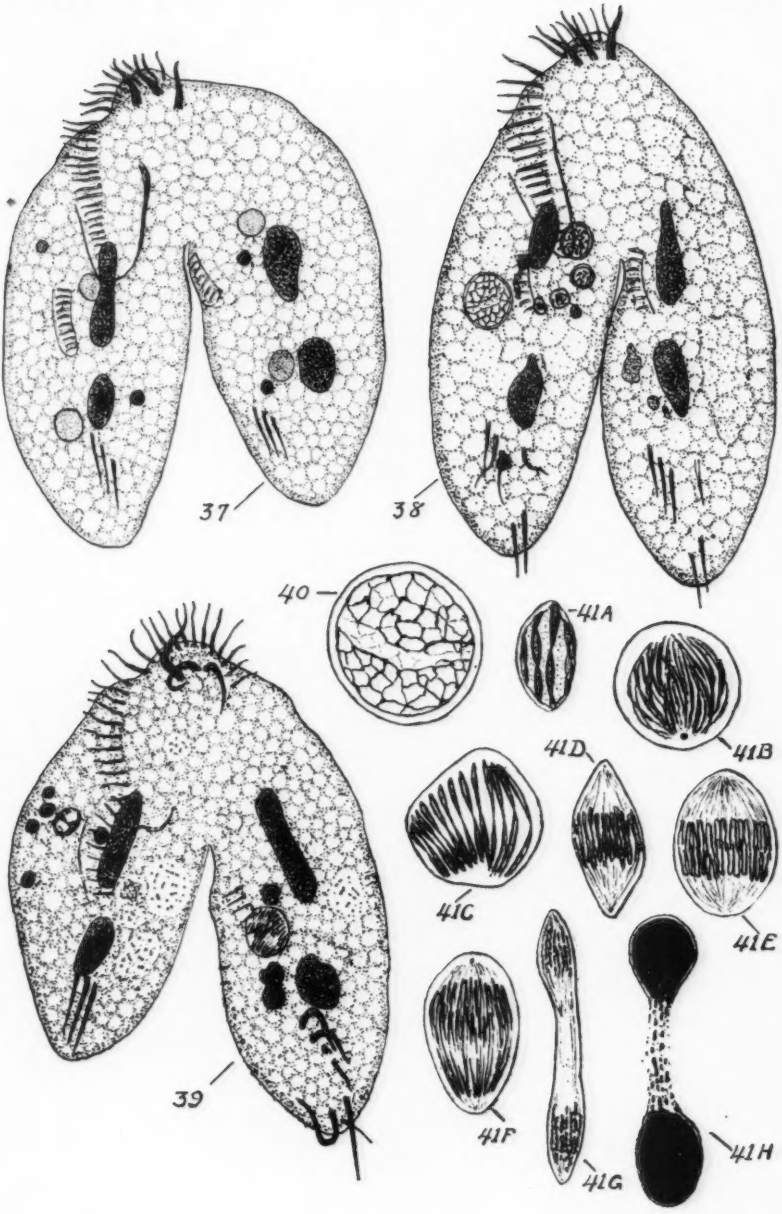

37
38
40
41A
41B
41C
41D
41E
41F
41G
41H
39

PLATE X.

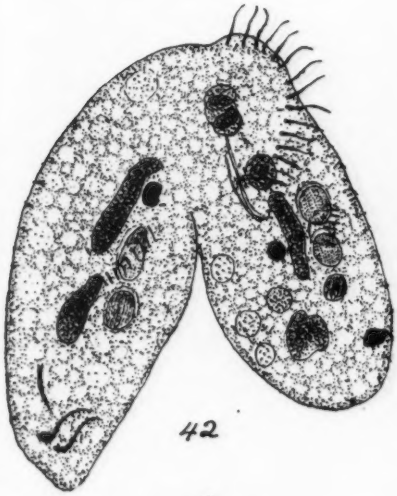
The Second Cleavage Division.

FIG. 42. Shows several prophases.

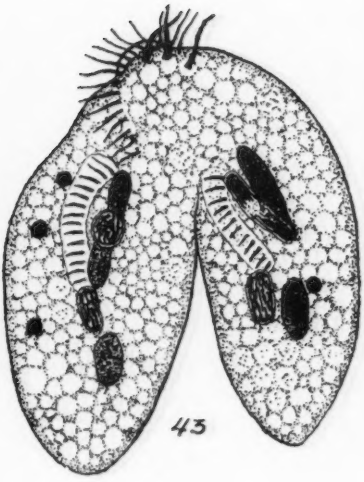
FIG. 43. In this pair a prophase and anaphase are visible in the left-hand individual, and a prophase and telophase in the right.

FIG. 44. A prophase and telophase may be seen in each of the members of this pair, and also several degenerating micronuclei which still persist from the maturation divisions.

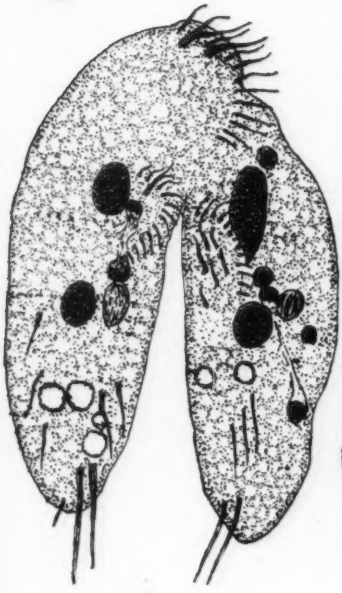
FIG. 45. The cleavage divisions are complete in the left-hand individual but a prophase of the second division is still to be seen in the other member.



42



43



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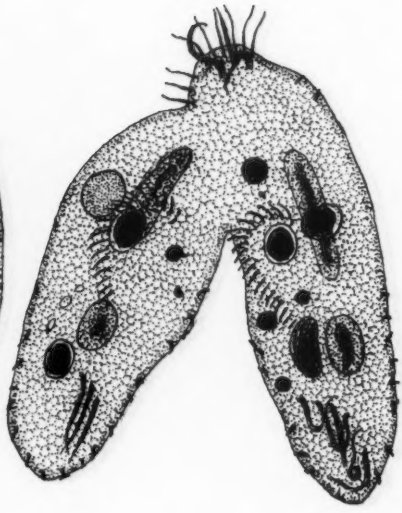

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PLATE XI.

The Second Cleavage Division and Early Stages of Reorganization.

FIG. 46 *A–M*. Stages of the second cleavage division, arranged consecutively.

FIG. 47. Shortly after the conclusion of the last cleavage division. Each individual contains several micronuclei, one of which is increasing in size and will give rise to the macronuclei of the reorganized exconjugant.

FIG. 48. The stage is similar to that in the preceding figure, except that the old macronuclei are becoming vesicular and are beginning to stain much more heavily than normally.

FIG. 49. A pair more completely fused than usual, and with the distribution of micronuclei irregular. The macronuclei are noticeably degenerating.

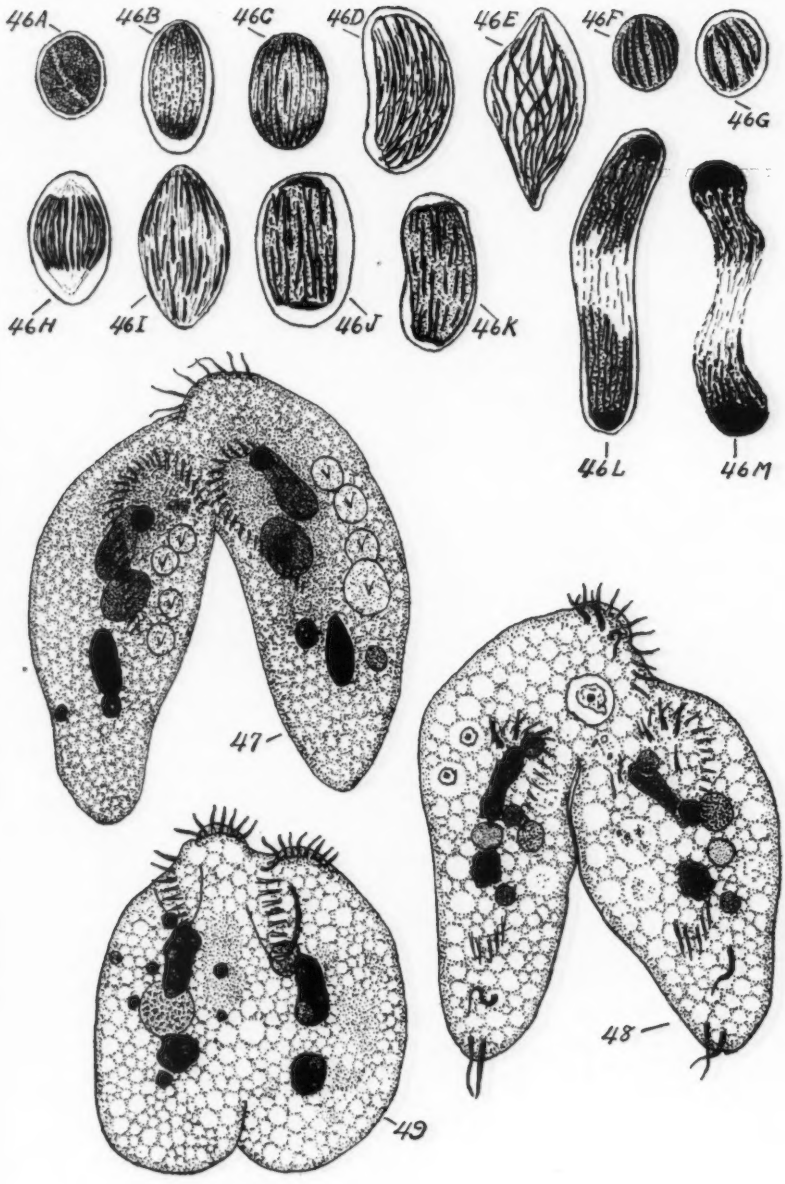

46A
46B
46C
46D
46E
46F
46G
46H
46I
46J
46K
46L
46M
47
48
49

PLATE XII.

Reorganization.

FIG. 50. A pair the members of which are about to separate. The fourth of the nuclei arising from the last cleavage has apparently degenerated in each individual, and the macronuclei have taken on the typical circular and vacuolated appearance which persists until their final degeneration.

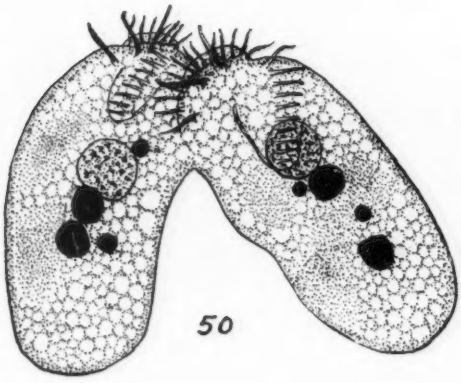
FIG. 51. Exconjugant shortly after separation. Typical of those individuals in which a third cleavage division has occurred.

FIG. 52. Exconjugant somewhat longer after separation than the one shown in Fig. 51.

FIG. 53. Exconjugant in somewhat more advanced stage than the preceding The remnants of the old macronuclei have finally disappeared.

FIG. 54. Reorganization in this individual has apparently been completed without preliminary division.

FIG. 55. One of the daughter individuals produced by the first division of an exconjugant. The macronucleus is in process of division by which the normal nuclear constitution will be finally restored.



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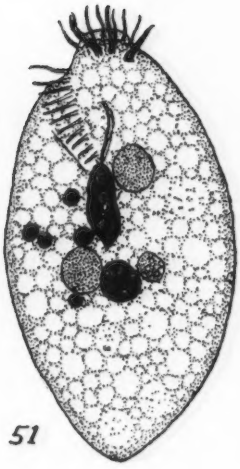

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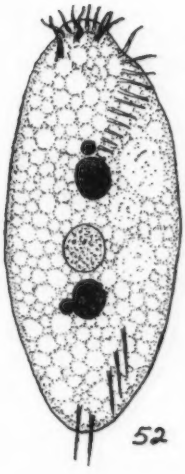

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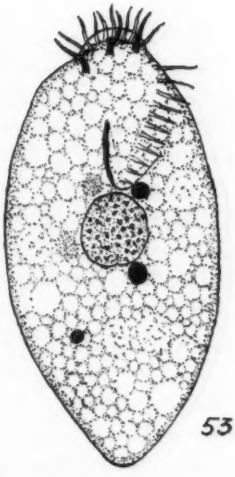

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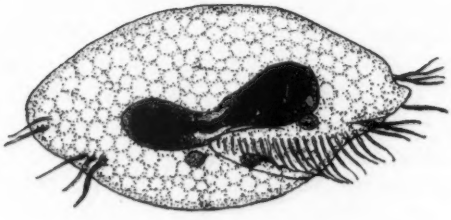

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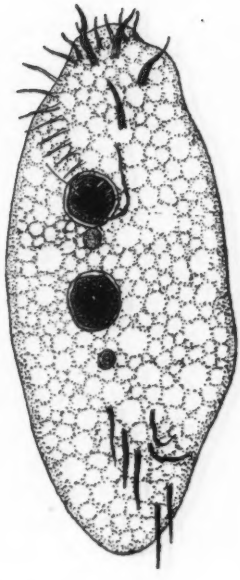

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